MICROBIOLOGICAL REVIEWS, Dec. 1989, p. 410–449
0146-0749/89/040410-40$02.00/0

Vol. 53, No. 4

Rotavirus Gene Structure and Function

MARY K. ESTES[1]* AND JEAN COHEN[2]

Division of Molecular Virology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030,[1] and Institut National de la Recherche Agronomique, Station de Recherche de Virologie et d'Immunologie Moleculaires, C.R.J. Domaine de Vilvert, 78350 Jouy-en-Josas, France[2]

INTRODUCTION

Rotaviruses, which form one genus of the family *Reoviridae*, are now recognized as the most important cause of severe viral gastroenteritis in humans and animals (see references 75, 100, 107, 147, 149, and 173 for reviews). These viral pathogens are more diverse than was originally thought, and this review highlights information learned during the past 5 years on the classification, biology, gene expression, and pathogenesis of the rotaviruses. Historical, epidemiological, and clinical features of rotavirus infections, methods of virus detection, and details of the replication cycle are considered only if they report significant new information relevant to the biology of these viruses. These topics are covered extensively in the reviews noted above and in others (16, 96, 101, 314, 337; M. K. Estes, *in* B. N. Fields, ed., *Virology*, 2nd ed., in press).

This review also highlights the usefulness of studying the rotaviruses as models to obtain basic information on protein processing, ribonucleic acid (RNA) replication, and viral morphogenesis in eucaryotic cells. Progress toward understanding what influences the genetic and antigenic variability of the rotaviruses and the outcome of rotavirus infections of the gastrointestinal tract is emphasized. This review was undertaken with the goal of identifying areas in which new research may be useful to further understand and help combat these viruses (and other members of the family *Reoviridae*) in the laboratory and in nature.

* Corresponding author.

ROTAVIRUS CHARACTERISTICS AND CLASSIFICATION

Morphologic and biochemical characteristics shared by members of the rotavirus genus include the following: (i) mature virus particles are nonenveloped and possess a multilayered icosahedral protein capsid, approximately 75 nm in diameter, composed of an outer layer, an inner layer, and a core; (ii) the virus genome consists of 11 segments of double-stranded RNA (dsRNA); (iii) particles contain an RNA-dependent RNA polymerase and other enzymes capable of producing capped RNA transcripts; (iv) virus replication occurs in the cytoplasm of infected cells; (v) the viruses are capable of genetic reassortment; (vi) virus cultivation in vitro is facilitated by treatment with proteolytic enzymes, which enhances infectivity by cleavage of the outer capsid polypeptide VP4; (vii) virus particles are formed by budding into the endoplasmic reticulum (ER), and enveloped particles are evident transiently at this stage of morphogenesis; and (viii) mature particles are liberated from infected cells by cell lysis. Only a few of the numerous isolates included in the rotavirus genus are known to possess all these characteristics. Instead, most isolates have been included in the genus on the basis of morphology, the presence of 11 segments of dsRNA, or antigenic cross-reactivity.

Until 1980, all rotaviruses were thought to have common antigens that were detectable by immunofluorescence, complement fixation, or enzyme-linked immunosorbent assays (ELISAs) (107, 353) and to fall into a limited number of species-specific virus serotypes. Recent studies have shown that neither of these early hypotheses is true. Instead, it is now known that (i) many isolates do not share cross-reacting antigens with the rotaviruses originally shown to cause gastroenteritis in the young (39a, 231, 249), (ii) many (at least six) human serotypes exist (Table 1), (iii) strains of animal and human origin occur within the same serotype (Table 1), and (iv) two genome segments encode neutralization antigens, and these segments can segregate (reassort) independently (153, 237).

These developments have emphasized the need for and importance of developing a serologic classification scheme for rotavirus isolates that allows for the presence of multiple groups of rotaviruses and for the existence of serotypes which cross species. This need has been addressed by a number of investigators (133, 154, 249, 280), but a uniform classification system remains to be established.

Although no classification system has been officially adopted, rotaviruses are classified serologically first into groups (or serogroups) containing viruses that share cross-reacting antigens detectable by serologic tests such as immunofluorescence, ELISA, and immunoelectron microscopy. Six distinct groups (A to F) of viruses have been described (39a, 231, 249). Group A, B, and C rotaviruses have been found in both humans and animals; group D, E, and F rotaviruses have been found only in animals (39a). Group A rotaviruses have clearly been established as causing severe diarrheal disease in the young, and they may cause disease more frequently in the elderly than previously recognized (155). Group B rotaviruses include viruses that have been associated with annual epidemics of severe diarrhea primarily in adults in China (56a, 160, 232, 319, 332). Group C viruses have been found in sporadic cases and outbreaks of diarrhea in piglets and children, but the severity and number of such infections are unclear (39a, 40a, 309, 342b). The clinical importance of the group B rotaviruses has begun to be studied owing to the recent establishment of rapid diagnostic tests (46, 231, 356). Available evidence indicates that infections with human group B rotaviruses have not been widespread outside China, but group B infections in animals are more common (40b, 232). The description of the non-group A rotaviruses is important, and the potential impact of these other groups of viruses to ongoing vaccine programs with the group A rotaviruses must be assessed. Because only one non-group A rotavirus strain has been successfully cultivated (a group C virus [292]), only limited information is available. Existing data suggest that the group B and C viruses have structural proteins similar to those of the group A viruses reviewed here (39, 46, 92, 103). Lack of reassortment of genes between viruses in different groups may be a useful criterion for taxonomic differentiation of virus groups. Unless noted otherwise, this review focuses on the group A rotaviruses.

Viruses within a serogroup are classified further into serotypes. Serotypes are defined by plaque reduction or fluorescent-focus reduction neutralization assays by using antisera to purified virus particles prepared in hyperimmunized animals. These assays measure the reactivity of antibody with the two outer capsid proteins (VP4 and VP7), which induce antibodies with neutralizing activity (151, 153, 237). In most cases the predominant antibody reactivity in hyperimmune serum is against the glycoprotein VP7. The simplest explanation for this is that VP7 makes up a greater percentage of the virion outer capsid of purified particles (see Table 4); alternatively, VP7 induces more specific antibodies than VP4 does following hyperimmunization regimens, possibly because VP4 is lost during preparation or storage of virus. The serotypes of viruses, originally defined by reciprocal neutralization assays with hyperimmune antisera, have now been confirmed to represent types of VP7; this was done by using monoclonal antibodies (MAbs) to specific epitopes on VP7 (26, 74, 120, 303, 330, 339, 340, 342). Previous references to rotavirus serotypes, therefore, really refer to viruses with specific VP7 types. The ability to successfully determine the VP7 type of viruses directly in stools by using MAb-based ELISAs has greatly simplified virus characterization.

In general, reactivities with VP4 are detected when two viruses generate a one-way (but not reciprocal) cross-neutralization with hyperimmune antiserum. The presence of higher proportions of antibody to VP4 in convalescent-phase antisera than in hyperimmune antisera and of a shared VP4 type between viruses with different VP7 types may explain why convalescent-phase antisera distinguish virus serotypes poorly (132). Support for this hypothesis is provided by recent estimates of protein-specific antibody levels in serum samples of volunteers to whom rotaviruses were administered (22a, 109, 301). Reagents for distinguishing VP4 types are not yet well characterized (45, 179, 304, 327, 328). Because serologic assays with hyperimmune antisera are poor discriminators of VP4 types and because the production of MAbs to characterize VP4 types has been slow, other approaches have been used to classify types of VP4. Gene 4 segments (which encode VP4) have been compared by hybridization and by nucleic acid sequencing. These comparisons suggest that at least nine types of VP4 exist (of which four occur in human viruses [Table 2]). Whether VP4 types (distinguished by these nonserologic methods) actually reflect different antigenic types remains to be determined.

Different virus isolates also can be distinguished by nonneutralizing epitopes located on the major polypeptide of the inner shell (VP6). Two such epitopes, now called subgroup antigens, were first described when human viruses were

TABLE 1. Classification of group A rotaviruses based on outer capsid protein VP7[a]

VP7 (G) serotype	Strain from following species of origin:	
	Human	Animal[b]
1	Wa, KU, RV-4, K8, M37, D, S12, Mont	
2	DS-1, S2, RV-5, RV-6, HN-126, 1076	
3	Ito, Yo, P, M, Nemoto, AU-1, RV-3, W178	Si/SA11, Si/RRV:1, Si/RRV:2, Po/CRW-8, Po/MDR-13, Po/AT/76, Ca/K9, Ca/CU-1, La/Ala, La/C-11, La/R-2, Mu/EB, Mu/EW, Eq/H-2, Eq/FI-14, Fe/Taka, Fe/2, Fe/3, Fe/22, Fe/97
4	Hochi, Hosakawa, St. Thomas-3, VA70, 57M	Po/Gottfried, Po/SB-1A, Po/SB-2, Po/BEN144
5		Po/OSU, Po/TFR-41, Po/EE, Eq/H-1
6		Bo/NCDV, Bo/UK, Bo/486, Bo/Rf, Bo/WC3
7		Ch/2, Ty/1
8	69M, B37	
9	WI-61, F45, AU32	
10		Bo/223
11		Po/YM

[a] Updated from reference 95 (with information from references 3, 62, 140, 150, 154, 180, 212, 214, 228, 229, 234a, 283, and 339).

[b] Abbreviations: Si, simian; Po, porcine; Bo, bovine; Eq, equine; Fe, feline; La, lapine; Ch, chicken (avian); Ty, turkey (avian); Ca, canine; Mu, murine. The year and country of origin are not indicated, because this information is not available for all strains.

differentiated by ELISAs. These reactivities were originally confused with neutralizing epitopes and were thought to be recognizing serotypes, but ultimately these epitopes were shown to be nonneutralizing and to be located on the inner capsid protein VP6 (174). Viruses can be characterized as possessing one, both, or neither of these epitopes with subgroup-specific (subgroup I or II) MAbs (137, 150, 323). Other epitopes not yet compared with the subgroup epitopes have also been found on VP6 (189, 190, 261). Characterization of virus strains by epitopes on this independent antigen can be useful for epidemiologic studies, for monitoring virus transmission, and for identifying natural reassortants.

Other studies of the antigenic properties of rotavirus isolates have shown that group determinants, or common determinants, are found on most (if not all) of the structural proteins of particles (95). This has been documented by showing that antisera (and some MAbs) specific for individual polypeptides cross-react with virus strains in all serotypes.

A simple and effective classification of any rotavirus strain seems possible by the use of a cryptogram (modified from that originally proposed by Rodger and Holmes [280]) that conveys the following information: group/species of origin/place of origin/strain designation/year/subtype antigens (to indicate the independent antigens encoded on different gene segments). The use of a single letter would simplify the notation. For example, the letter P (to denote the protease-sensitive outer capsid protein VP4 that is the hemagglutinin in some strains) and the letter G (to denote the outer capsid glycoprotein VP7) were proposed at the International Congress of Virology, Edmonton, Alberta, 1987. The VP6 subgroup antigen would be abbreviated S_I or S_{II}, as already accepted in the literature (352). An example of this scheme applied to the best-characterized rotavirus strain, the simian rotavirus SA11, is A/SI/S. Africa/SA11/58/G_3,P_2,S_I. In abbreviated form, this strain would be referred to as A/SI/SA11/G_3,P_2,S_I or A/SA11/G_3,P_2,S_I. This indicates that SA11 contains a type 3 VP7, a type 2 VP4, and a subgroup I VP6. Designation of the SA11 VP4 as a type 2 is used as an arbitrary example. The designation of groups and numbering of subtype antigens (VP4 and VP7 types) must be standardized by an international committee of veterinary and medical researchers to facilitate scientific communication and the development and exchange of standard reagents.

The preparation of polyclonal typing serum to classify the distinct types of VP4 and VP7 (as has been accomplished for the influenza virus H and N subtypes) would certainly represent a major step in simplifying the characterization of both existing and future virus isolates. However, whether such antiserum can be produced and whether classification by VP4 and VP7 is feasible and useful remains to be demonstrated. Possible problems with this approach are that it remains unknown whether infection with a virus with specific P and G types correlates with protection from a second challenge. In addition, certain combinations of VP4 and VP7 may alter the antigenic structure of these individual proteins (56) and some epitopes on VP7 are shared by viruses with different VP7 types (73, 157). However, if such changes are subtle or detectable only with MAbs, they may not significantly affect the typing with monospecific polyclonal antisera.

VIRION STRUCTURE

The morphologic appearance of rotavirus particles is distinctive. Intact virus particles resemble a wheel, with short spokes and a well-defined rim, when examined by negative-stain electron microscopy (EM). The name rotavirus (from the Latin rota, meaning wheel) was suggested on the basis of this characteristic (106). Three types of particles (double-shelled, single-shelled, and core) are often observed by EM (Fig. 1). SA11 double-shelled particles are 76.5 nm in diameter, single-shelled particles are 70.5 nm in diameter, and cores are 50 nm in diameter. Single-shelled particles and cores can be produced by chemical disruption of double-shelled or single-shelled particles, respectively. It is unknown whether the single-shelled or core particles are identical to subviral particles synthesized during virus replication.

Early structural studies of rotavirus particles agreed that these particles possessed icosahedral symmetry, but the ultrastructure of the virus was controversial. For example, the triangulation number (T), which designates the relationship between the neighboring fivefold axes of the icosahedral surfaces, was initially reported to vary from 3 to 16 (90, 181, 205, 281). The application of new techniques (which avoided double-sided images produced by negative staining) to the study of rotavirus structure has recently resolved these earlier discrepancies. Roseto et al. (281) studied the structure of single-shelled rotaviruses by using a freeze-drying technique and reported the existence of 132 capsomeres arranged in a skew symmetry with $T = 13$. They also showed that the outer layer contained small holes that corresponded one-to-one with holes in the inner capsid. This structure was subsequently confirmed for the inner capsid by Ludert et al. (200), using the same technique and chemically disrupted particles.

The three-dimensional structure of double- and single-shelled rotavirus particles, determined at a 4-nm resolution

TABLE 2. Tentative typing of group A rotaviruses based on outer capsid protein VP4[a]

Inferred VP4 (P) type	Strain from following species of origin:	
	Human	Animal
1		Si/SA11 4fM, Bo/486, Bo/NCDV
2		Si/SA11
3		Si/RRV:2
4	RV-5, DS-1	
5		Bo/UK
6	M37, RV3, 1076	ST3, Po/Gottfried
7		Po/OSU, Po/SB-1A
8	Wa, KU, P, VA70	
9	K8	

[a] The numbering of types of VP4s shown here is arbitrary. These VP4 types have been defined by nucleic acid hybridization and sequence studies (see Table 3 for references). Whether such differences reflect antigenic differences is uncertain.

by using cryoelectron microscopy and image-processing techniques (264), provides new insights into the virion structure (Fig. 2). These studies have unequivocally established a $T = 13\ l$ (levo) icosahedral surface lattice for the two outer layers. A distinctive feature of the virus structure is the presence of 132 large channels spanning both shells and linking the outer surface with the inner core; 120 channels are along the six-coordinated centers and 12 are along the five-coordinated centers.

Three types of channels can be distinguished on the basis of their positions and sizes. The type I channels are those running down the icosahedral fivefold axes, the type II channels are those on the six-coordinated positions surrounding the fivefold axes, and the type III channels are those on the six-coordinated positions around the icosahedral threefold axes. Type III channels are about 14 nm in depth, and they are about 5.5 nm wide at the outer surface of the virus. Going into the particle, these channels constrict and then widen, and their maximum width is close to the surface of the inner-shell proteins. Similar features and dimensions are seen in the other two types of channels, except that type I channels have a narrower (ca. 4.0-nm) opening at the outer surface of the virus. The biological role of these channels is not yet clear, but it is likely that they are involved in importing the metabolites required for RNA transcription and exporting the nascent RNA transcripts for subsequent virus replication processes.

These studies of non-protease-treated virus particles also revealed structural features not seen previously (264). Sixty spikes, at least 4.5 nm (4.5 to 6.0 nm) in length and each with a knob at its distal end, have been shown to extend from the smooth surface of the outer shell (Fig. 1 and 2). These protein spikes are situated at an edge of the channels

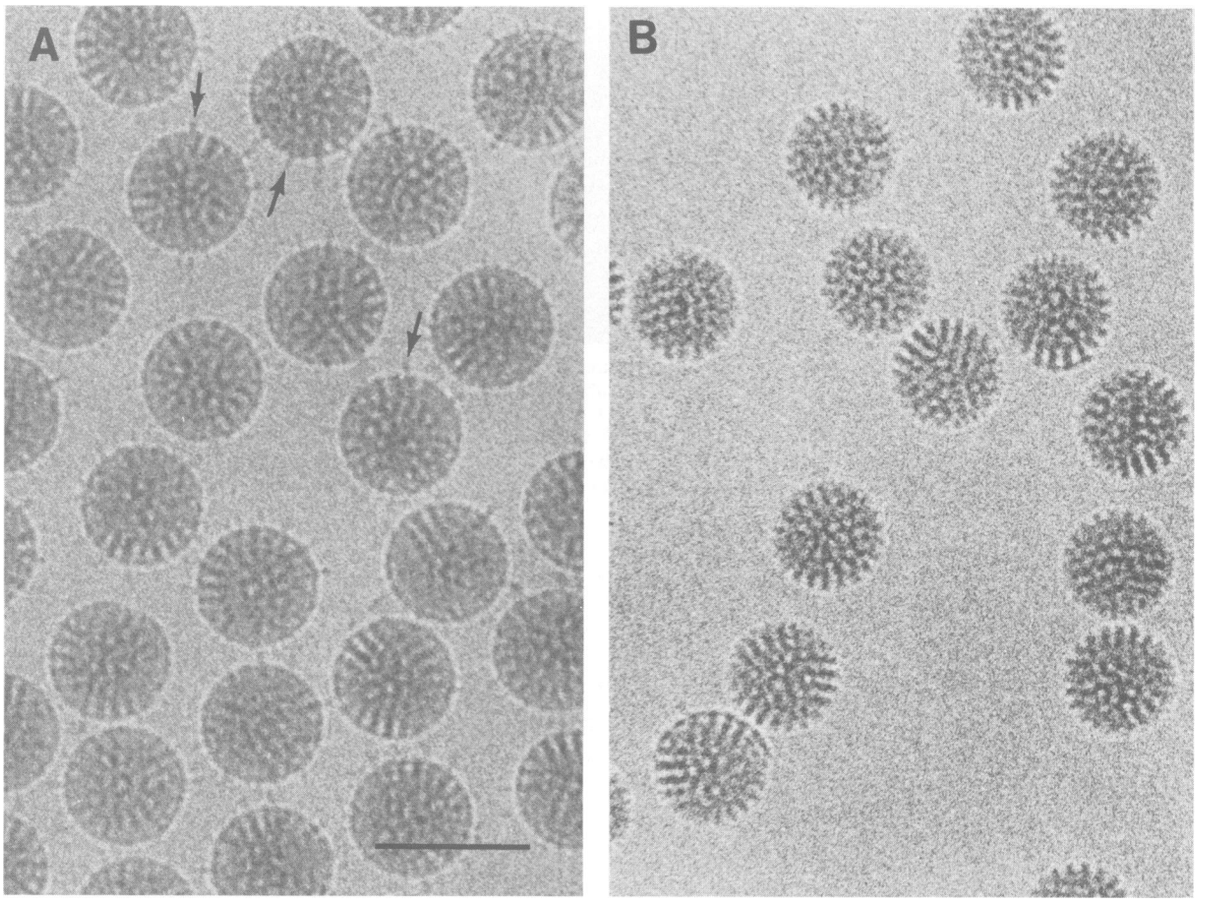


FIG. 1. Electron micrographs of (A) double-shelled and (B) single-shelled SA11 particles embedded in vitreous ice and examined by cryoelectron microscopy. Arrows indicate the spikes visible on the outer surface of some of the virions. Bar, 100 nm. Reprinted from the *Journal of Molecular Biology* (264) with permission of the publisher.

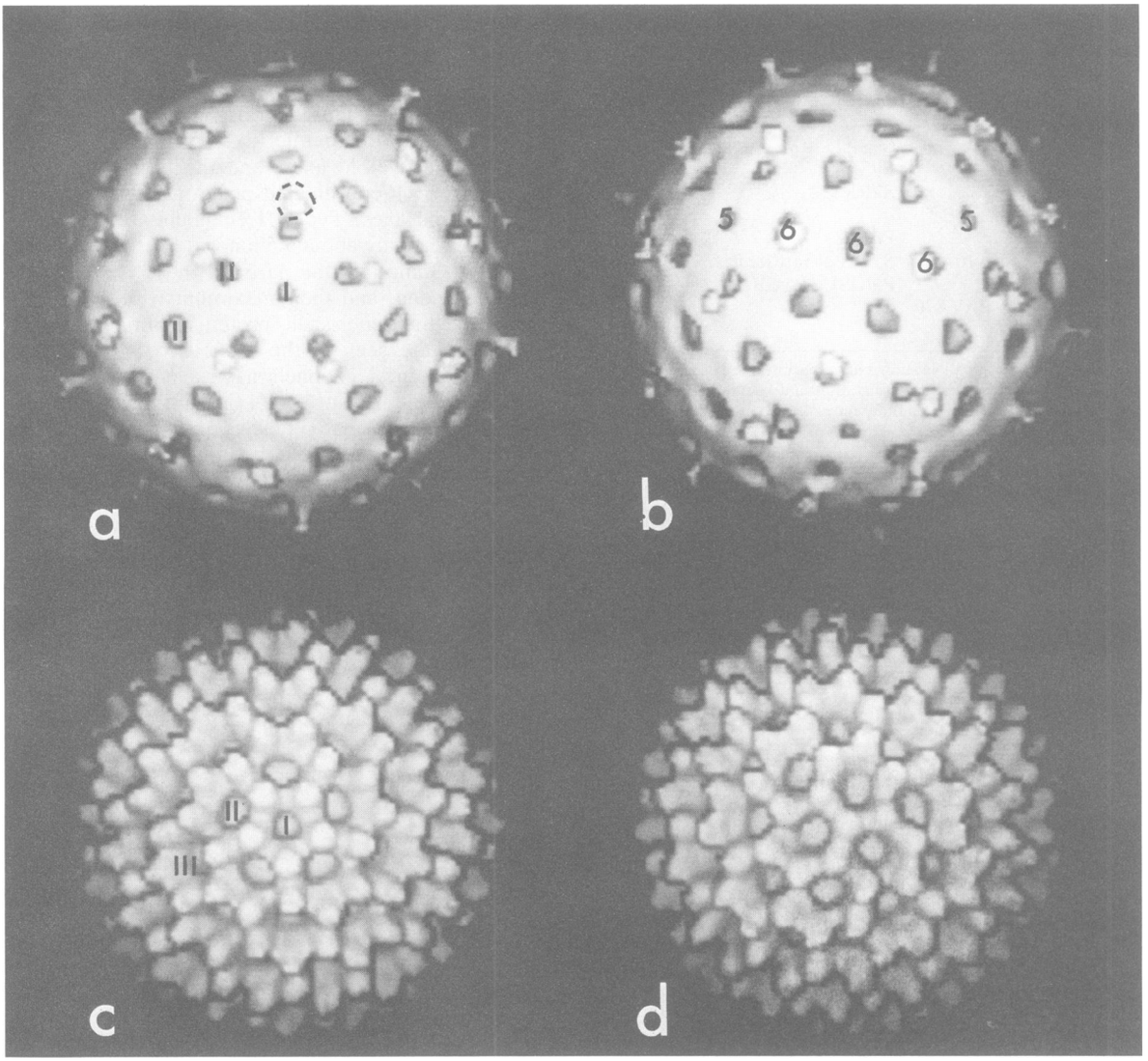


FIG. 2. Surface representations of the three-dimensional structure of double- and single-shelled rotavirus particles. The particles are shown along the icosahedral fivefold axis (a and c) and along the icosahedral threefold axis (b and d). In panels b and d, a pair of neighboring fivefold axes (designated as 5) and six-coordinated positions (designated as 6) are indicated to illustrate $T = 13\ l$. Three types of channels (I, II, and III in panels a and c, showing one of each type) are found at all five- and six-coordinated positions spanning the outer- and inner-shell proteins. One of the protein spikes situated at the edge of type II channels surrounding the fivefold positions in double-shelled particles is highlighted with a dashed circle. Reprinted from the *Journal of Molecular Biology* (264) with permission of the publisher.

surrounding the fivefold icosahedral axes, and they apparently are composed of dimers of the hemagglutinin (VP4) known to be present on the outer capsid of the particles (B. V. V. Prasad, J. W. Burns, E. Marietta, M. K. Estes, and W. Chiu, submitted for publication). Examination of the spike structure in protease-treated particles will be of interest. It is noteworthy that new studies have shown that reovirus particles have long fibers topped with knobs extending from the surfaces of virions and that these fibers have been shown to be composed of the sigma 1 protein, which is the hemagglutinin and a major determinant of virulence (115).

The distinctive morphology of rotaviruses is easily seen by conventional EM, and for this reason, EM remains the standard against which new diagnostic techniques (ELISAs, latex agglutination, etc.) are compared. In addition, EM (in conjunction with either immunologic analyses or RNA analyses) continues to be useful for detecting the non-group A rotavirus strains that do not share any antigenic cross-reactivities with previously identified strains (see above). Since EM remains so critical for diagnosis, it is important to know that the detection of rotaviruses by EM can be influenced by the method used for staining. For example, negative staining with uranyl acetate or with phosphotungstic acid at low pH (pH 4.5) allows detection of all rotavirus strains; however, staining with phosphotungstic acid at neutral pH may result in removal of the outer capsid of particles and complete disintegration of virus particles, particularly with non-group A rotaviruses (233, 320).

GENOME STRUCTURE

The viral genome of 11 segments of dsRNA is contained within the virus core capsid. The segments range in size from

667 (segment 11) to 3,302 base pairs (segment 1), with the total genome containing approximately 18,522 base pairs (Table 3). This number, compiled from sequence data of segments from different virus strains, agrees closely with the genome size (18,680 base pairs) determined by EM measurements (279a).

Hydrodynamic studies of the flexibility or stiffness of isolated rotavirus RNA segments in solution have indicated that these RNA segments cannot be packaged into the rotavirus capsid unless intimate protein-RNA interactions take place. In solution, these RNA molecules possess a "wormlike" or flexible cylinder structure; as an example, RNA segment 1 (3,302 base pairs and a contour length of 928 nm) theoretically cannot be bent into a capsid of 50 nm as a free molecule because the persistence length is 112.5 nm (172). Therefore, to obtain RNA flexibility, one has to assume that intimate protein-RNA interactions occur in the virion to induce the needed bending and packaging of the dsRNA segments into the virus capsid. The proteins directly responsible for segment packaging remain unclear. The structural proteins present in core particles (VP1, VP2, and VP3) are obvious candidates, but nonstructural proteins may also play a scaffolding role. Deproteinized, purified rotavirus dsRNAs are not infectious, reflecting the fact that virus particles contain their own RNA-dependent RNA polymerase required to transcribe the individual RNA segments into active messenger RNAs (mRNAs) (66).

Sequences of Rotavirus RNA Segments

The first nucleotide sequences of the rotavirus RNA segments were determined following the development of rapid deoxyribonucleic acid (DNA) sequencing methods and of methods to synthesize dsDNA from templates of either single-stranded viral RNA transcripts or from genomic dsRNA. Once initial sequence information for any RNA segment was obtained, direct dideoxy-chain termination sequencing with reverse transcriptase and oligonucleotide primers with mRNA (or denatured dsRNA segments) often bypassed the need for additional molecular cloning and facilitated the rapid acquisition of comparative sequence information from numerous virus strains. The nucleotide sequences of the 11 RNA segments from different virus strains are known (Table 3), and the complete nucleotide sequence of one group A rotavirus (SA11) should be established soon. Sequence data and direct measurements of the lengths of rotavirus dsRNA molecules by EM were used to determine the translation, or axial distance, between base pairs in RNA duplexes. The rise per base pair in a helix of dsRNA was found to be 0.28 ± 0.011 nm, indicating that dsRNA contains from 1.16 to 1.17 times more nucleotides per unit length than does dsDNA (332a). The properties of each RNA segment and its encoded polypeptide(s) are described below.

General Primary Structure of Genome Segments

The nucleotide sequence data (Table 3) reveal general features about the structure of each of the 11 genome segments and sequences common to all RNA segments (see Fig. 3 to 8). Each RNA segment starts with a 5′ guanidine followed by a set of conserved sequences that are part of the 5′ noncoding sequences; an open reading frame coding for the protein product follows, and another set of noncoding sequences, which contains a different subset of conserved 3′-terminal sequences and ends with a 3′-terminal cytidine, is found after the stop codon. The 5′-terminal consensus sequence 5′-GGC(A/U)(A/U)U(A/U)A(A/U)(A/U) is found only once (in segment 4, base pairs 2166 to 2177) in a position other than at the termini of the other known sequences. The 3′-terminal consensus sequence is U(G/U)(U/G)(G/U)(A/G)CC-3′. The complementary sequence of the 3′-terminal consensus sequence (UGUGACC-3′) is also found in segment 5 (positions 1513 to 1519). The lengths of the 3′ and 5′ noncoding sequences vary for different genes. However, these lengths are conserved among strains for a given gene, with the exception of the 3′ end of segment 7 (134 bases for SA11 and 112 bases for bovine UK viruses) and segment 10 (from 182 to 186 bases for five different virus strains). There is no preferential use of stop codons, and a polyadenylation signal is not found at the 3′ end of the genes. Most of the sequenced segments (except gene 11) possess only one long open reading frame. The first initiation codon (in all genes except 7, 9, and 11) is a strong initiation codon based on Kozak's rules (185, 186). For genes 7 and 9, the second in-phase AUG is the favored initiation sequence. For gene 7, it is unknown which AUG is used. Some evidence (see below) indicates that gene 9 is a bicistronic gene (49). Gene 11 contains a second in-frame open reading frame and also an out-of-frame open reading frame that are conserved in all of the segment 11 sequences found to date. The initiation codon for the second out-of-frame open reading frame is a favorable one, but it is not known whether this second reading frame is used.

All of the rotavirus gene sequences are A+T rich (58 to 67%), and the codon usage is biased against CGN and NCC codons, as in many eucaryotic and other viral genes (260). The RNA segments are base paired end-to-end, and the plus-sense strand of the genomic dsRNA contains the capped 5′ sequence $m^7GpppG^{(m)}GPy$ (161, 218). Similar features of the RNA termini (capped structures and 5′ and 3′ conserved sequences) are found in the primary structures of the genome segments of other viruses (e.g., reovirus, cytoplasmic polyhedrosis virus, and orbivirus) in the family *Reoviridae* (116, 187, 222), and 5′ and 3′ conserved terminal sequences are also found in other virus families with segmented genomes (*Orthomyxoviridae*, *Arenaviridae*, and *Bunyaviridae*). Therefore, these terminal sequences are thought to contain signals important for genome transcription, replication, and possibly assembly of the viral genome segments.

Secondary Structure of Rotavirus Genome Segments or Viral Transcripts

Potential regions of intramolecular base-paired (secondary) structures of the plus strand involving terminal regions have been described for gene segments 4, 10, and 11 (162, 171, 244). These structures are hypothesized to influence gene expression during either RNA replication or translation, and they have caused difficulties in obtaining full-length complementary DNAs (cDNAs). Examination of terminal sequences of each known gene sequence for direct and inverted repeats shows that several segment ends contain regions with statistically significant similarities. These regions could represent gene duplication and aberrant transcription events of both strands of the genome. However, the distribution of these patterns does not lead to a selective association process that could explain gene reassortment, a finding also previously described for reoviruses (7). Thus, it can be hypothesized that the reassortment of the 11 segments probably involves RNA-protein interactions.

TABLE 3. Nucleotide sequences of rotavirus RNA segments[a]

RNA segment	RNA size	Protein	Strain(s)	Reference(s)
1	3,302	VP1	A/Bo/Rf	66a
2	2,687	VP2	A/Bo/Rf	188
3	2,591	VP3	A/Si/SA11	Liu and Estes, in press
4	2,362	VP4	A/Si/SA11 4fM	195, 196
			A/Bo/486	262
			A/Hu/RV-5	170
			A/Si/RRV	204
			A/Hu/Wa, DS-1, P, VA70, M37, 1076, McN13, ST3	126
			A/Si/SA11	225a, 235
			A/Bo/NCDV	235
			A/Bo/UK	171
5	1,581	NS53	A/Bo/Rf	38
6	1,356	VP6	A/Si/SA11	36, 99
			A/Hu/Wa	36
			A/Bo/Rf	68
			A/Hu/S2	148
			A/Hu/1076	128
			A/Eq/FI-14	128
			A/Po/Gottfried	128
7	1,104	NS34	A/Si/SA11	31
			A/Bo/UK	346
			A/Po/OSU	284
8	1,059	NS35	A/Si/SA11	31
			A/Bo/UK	80
			A/Po/OSU	284
9	1,062	VP7	A/Si/SA11	10, 32
			A/Bo/UK	87
			A/Hu/Hu5	83
			A/Hu/Wa	206, 276
			A/Hu/S2	142
			A/Bo/NCDV	122
			A/Bo/Rf	54
			A/Po/OSU	125
			A/Si/RRV	135
			A/Hu/D, MO, M37, DS1, HN126, P, VA70, S73	135
			A/Po/OSU	284
			A/Po/Gottfried	130
			A/Po/YM	283
			A/Hu/KU	326
			A/Hu/69M, W161	134
			A/La/Ala, R-2, C11	234a
10	751	NS28	A/Si/SA11	35
			A/Hu/Wa	244
			A/Bo/UK	21, 345
			A/Bo/NCDV	263
11	667	NS26	A/Si/SA11	225, 349
			A/Hu/Wa	163
			A/Bo/UK	345

[a] Compiled April, 1989. The gene-protein assignments are for SA11 (see Table 4), and sequences of the cognate gene in other strains are listed on the basis of homology with the SA11 gene. For some strains, this represents a different RNA segment based on electrophoretic migration on polyacrylamide gels. For example, the SA11 genome segment 8, the bovine UK genome segment 7, and the rhesus rotavirus genome segment 9 encode NS35, whereas SA11 genome segment 9, UK genome segment 8, and RRV genome segment 7 encode VP7 (80, 123, 124, 208, 217). Such shifts are most common for genome segments 7, 8, and 9. The order of migration of segments 10 and 11 is also changed between viruses with long and short RNA patterns. The size of each segment is given only for the first virus listed and may differ for the same segment in other virus strains. Segment size differences have been reported for genome segments 4, 7, 10, and 11, and these differences may be due to genetic drift, rearrangements of sequences within a segment, or sequencing errors (see text).

Evolution of the Rotavirus Genome

Comparisons of the sequences of individual segments of different group A rotaviruses have shown that changes occur through genetic shift (genome reassortment) and drift (sequence changes within segments) via mechanisms analogous to those seen with the type A influenza viruses (2). In addition, changes apparently occur by another mechanism (rearrangements within a genome segment [described below]). A comparison of sequences of the gene encoding the neutralization glycoprotein VP7 of 27 human and animal viruses which each have a type 3 VP7 suggests that there are species-specific sequences in this gene (234a), as well as serotype-specific regions (see below and Fig. 6). The identification of species-specific, unique amino acid sequences in this gene suggests that the VP7 gene is involved in interspecies reassortment with low frequency in nature (234a). The biologic properties and antigenic composition of particles may be altered by such genomic evolution, but this is only beginning to be understood. Interesting recent results indicate that the antigenic composition of particles may be influenced by the interactions of specific combinations of the two outer capsid proteins, by trypsin treatment of particles, or by oligosaccharide addition at different sites on the outer capsid glycoprotein (47, 56, 72, 203, 302).

Genome Analysis for Virus Detection

Rotaviruses are the only mammalian agents known to contain 11 segments of dsRNA. In most cases, the genome of the group A viruses is composed of four high-molecular-weight dsRNA segments (segments 1 to 4), five middle-sized segments (segments 5 to 9) including a distinctive triplet of segments (segments 7 to 9), and two smaller segments (segments 10 and 11). Analysis of the genome pattern of virus specimens is relatively easy and rapid; therefore, this technique has become a useful and popular procedure for virus detection and for molecular epidemiology studies (see references 53 and 96 for reviews).

Although in most cases the basic pattern described above is seen, RNA analyses have shown that some rotaviruses have distinct genome migration patterns. Viruses with such distinct patterns have been characterized as either non-group A viruses or group A viruses that contain rearrangements within individual genome segments (see below). Since these distinct RNA patterns can arise by different mechanisms, RNA profiles cannot be used as the sole criterion for classification of a virus strain in a specific group (i.e., A to F). Instead, RNA profiles combined with group A ELISA reactivity should be used for preliminary characterization of non-group A rotaviruses (96, 249, 309). For viruses within a specific group, RNA patterns cannot be used to classify

them into different serotypes or subgroups (1, 22, 41, 117, 229, 298, 325, 333, 341). However, because RNA patterns usually remain constant for individual virus strains, RNA analysis is useful for molecular epidemiology studies to monitor virus outbreaks and transmission.

The electrophoretic migration of the same gene in different virus strains often shows heterogeneity. In contrast, the gene sequence data show that the same genes from different strains almost always contain the same number of nucleotides. This suggests that the heterogeneity in RNA segment mobility, observed among the cognate RNA segments of different virus strains, is attributable to sequence differences and secondary structure (which remain during electrophoresis of the segments). Recent longitudinal surveillance studies suggest that correlation of RNA patterns with epitopes on specific proteins is possible within limits of time and location; confirmation of this awaits more extensive antigenic and RNA analyses of a large number of rotavirus strains isolated from distinct locations and in different years (70, 144, 230; D. O. Matson and M. K. Estes, unpublished data).

Genome Rearrangements

Analyses of some group A rotaviruses with atypical RNA profiles on gels have resulted in the recognition that rearrangements within genome segments can occur. In viruses with genome rearrangements, normal RNA segments are missing in an electrophoretic profile, and these are replaced by additional, more slowly migrating bands of dsRNA. These new bands usually represent concatemeric forms of dsRNA containing sequences specific for the missing RNA segments (158, 215, 250, 256, 325). Viruses with such genome rearrangements have been isolated from immunodeficient, chronically infected children (250), asymptomatically infected immunocompetent children (24), and animals (calves [256], pigs [23], or rabbits [325, 335]). They have also been obtained after serial in vitro passage of a tissue-culture-adapted bovine rotavirus at high multiplicity of infection (158). Isolates with rearrangements in segments 5, 6, 8, 10, and 11 (27, 158, 159, 215, 250, 256, 325) have been characterized, with the greatest number having rearrangements in segment 11. In retrospect, it is possible that short and super-short RNA patterns reported for human viruses reflect rearrangements in genome segment 11 (81). In fact, determination of gene 11 sequences from these strains has shown that they contain insertions of A+T rich 3′ noncoding regions which are not significantly homologous to each other, to other parts of gene 11, or to other rotavirus genes that have been sequenced (236a). These appear different from rearrangements described below.

Characterization of viruses containing rearranged genome segments has shown that these viruses are often not defective and that the rearranged segments can reassort and replace normal RNA segments structurally and functionally (4, 27, 131, 215). Biophysical characterization of such particles has shown that up to 1,800 additional base pairs can be packaged in particles without causing detectable changes in particle diameter or apparent sedimentation values. The density of particles containing rearranged genomes was increased, and the change in density was directly proportional to the number of additionally packaged base pairs (220). These results indicate that rotaviruses have considerable capacity to package additional genomic RNA, and one wonders what the upper limit of this capacity might be. Although a total of 11 RNA segments (or rearranged bands) are invariantly packaged, there seems to be much less constraint on the length of individual RNA segments assembled into the maturing virus particle.

In most cases, the profiles of virus-specific proteins in cells infected with rotaviruses with rearranged genomes are similar to those seen in cells infected with standard rotavirus strains (4, 27, 215, 256, 325). This indicates that the rearrangement of the segment-specific sequences apparently has left the normal reading frames and their expression unaltered.

This hypothesis has been confirmed by analyses of several rearranged genome segment 11 sequences. In two cases, the rearranged segment 11 consists of a partial duplication of segment 11-specific sequences, with maintenance of the open reading frame for the protein product from the first initiation codon and with the duplicated sequences lacking the initiation codon to reinitiate translation. In addition, the conserved 5′- and 3′-terminal sequences are present at the ends of the rearranged segments, but these are not present in the middle of the gene upstream from the duplicated sequences (124a; M. A. McCrae, personal communication). Other cases of rearrangements have apparently led to the abolition or extension of the normal reading frame, with the consequence that no protein or extended novel protein product is made (158); the sequence of one of these RNAs shows a duplication that extends "in-frame" of the normal reading frame (M. A. McCrae, personal communication). In another case, partial duplications and deletions (with the appearance of a possible new open reading frame) in a rearranged segment have been found (129a). Rearrangements leading to nonfunctional gene products probably would not have been identified by present assays.

One proposed mechanism for these rearrangements that is compatible with the available data is that at various stages of initial transcription, the virion-associated RNA polymerase falls back or jumps to a second site on its template to reiterate part of it in the transcript (124a, 129a, 159). Such copy choice mechanisms have been documented to explain production of deletion-defective particles of vesicular stomatitis virus and coronavirus recombinants (192a, 204a). For rotaviruses, these aberrant replicative events must be rare and the polymerase apparently maintains template specificity, because the formation of mosaic gene structures has not been seen. However, such rearrangements have not been seen in mRNA synthesized in vitro with the endogenous RNA polymerase, possibly because the correct conditions to synthesize large amounts (or to detect few copies) have not yet been tested. Without such evidence, one must also consider that these rearrangements might occur during replication of the RNA (synthesis of the negative strand off the mRNA template). Similar rearrangements have been described for the orbiviruses (85, 272). Therefore, genome rearrangements may be operative to a much greater extent than was previously envisaged as a mechanism of evolution of rotaviruses and other dsRNA viruses. It remains unknown whether such rearrangements also affect antigenic diversity (perhaps by altering the virion structure) or pathogenic properties of viruses (perhaps by altering the growth properties of viruses).

GENE-CODING ASSIGNMENTS

The gene-coding assignments and known properties of the proteins encoded in each of the 11 genome segments are now fairly well established (Table 4). These assignments have been determined by in vitro translation studies with mRNA or denatured dsRNA (81, 207, 208, 217, 307) and by analyses

of reassortant viruses (123, 168, 194, 237–239, 341). The information on SA11 serves as the basis for comparative studies with other rotavirus strains. Such comparisons have shown that the absolute order of migration of a gene coding for a particular protein (cognate gene) may differ for different virus strains, and so gene assignments cannot be based on RNA patterns alone (see Table 3, footnote *a*). Instead, identification of cognate genes must be based on hybridization with gene-specific probes (22b, 79, 101), analysis of reassortants, or protein identification based on biochemical or immunologic identification of the protein translated in a cell-free system programmed with mRNA specific to the gene. The ability to directly obtain sequence information from dsRNAs or ssRNAs (18, 135, 136) and the accumulating nucleic acid sequence data bases also make it possible to identify cognate genes solely on the basis of sequence homology.

The rotavirus genes code for structural proteins found in virus particles and for nonstructural proteins found in infected cells but not in mature particles. The consensus is that the protein products (VP1 to VP4, VP5*, VP6, VP7, VP8*) of six of the genome segments are structural proteins found in virus particles and that the other five genome segments code for nonstructural proteins. It remains unknown whether the additional open reading frames found in genome segment 11 code for other proteins.

Early studies often contained seemingly conflicting conclusions concerning the numbers and locations of the rotavirus proteins. Many of these were resolved, as reviewed elsewhere (100, 149), when it was recognized that posttranslational modifications (glycosylation, trimming of carbohydrate residues, and proteolytic cleavages) occur following polypeptide synthesis. In addition, strain variations (such as the presence of more than one glycosylation site on VP7 in some bovine and human rotavirus strains) have been clearly shown (183, 184, 294) (see below), and these provide explanations for other discrepancies.

The nomenclature of the viral proteins has recently been changed (194). We originally proposed the designation of the SA11 structural proteins as viral protein (VP) followed by a number, with VP1 being the highest-molecular-weight protein, and proteins generated by cleavage of a larger precursor were indicated by an asterisk (VP4 is cleaved to produce VP5* and VP8* [11, 97]). Our initial studies failed to identify a protein product from gene segment 3, and the protein product of gene segment 4 was called VP3 (208). When recent studies showed that the protein of gene segment 3 is a structural protein located in the inner core, for consistency we called the gene 3 product VP3 and renamed the gene 4 product VP4 (194). This new nomenclature is used throughout this review and will be used in all our future publications, but it should be realized that much of the literature on "VP3" up to and including 1988 refers to the gene 4 product.

Our naming the gene 3 protein VP3 brings into agreement our nomenclature system for SA11 with that for the UK virus for the products of genes 1 to 4 as designated by McCrae and McCorquodale (217) and for several human rotavirus strains (294). Our finding that the four largest RNA segments code for structural proteins confirms the earlier reports of others (149, 307) and also requires changes in another nomenclature system, in which the nonstructural proteins were called NCVP1 to NCVP5 and the gene 3 product was called NCVP1 (11, 33). We continue to designate nonstructural proteins by NS followed by a number indicating their apparent molecular weight (in thousands) seen following electrophoresis in denaturing polyacrylamide gels.

STRUCTURE AND FUNCTION OF ROTAVIRUS PROTEINS

Core and Inner Capsid Proteins

VP1. VP1 is encoded by genome segment 1 in all viruses studied to date, and it is one of three proteins (VP1, VP2, and VP3) that make up the rotavirus core particles. Information on these core proteins currently is limited. The deduced amino acid sequence of VP1 of a bovine rotavirus strain indicates that this protein migrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to its calculated molecular weight of 124,847 and that it is a relatively hydrophobic and slightly basic protein (66a). A small number of VP1 molecules (ca. 2% of the virion mass) are present in virions, suggesting that this protein does not have an important structural function but, rather, that it may be part of an enzymatic complex. Consistent with this idea, homology searches of the protein data base have revealed similarities with a family of RNA polymerases of RNA viruses (GDD consensus sequence in Fig. 3). This putative functional site and larger regions of amino acid similarity with VP1 are also present in the reovirus lambda 3 protein (amino acids [aa] 585 to 739) and in the bluetongue virus P1 protein (aa 667 to 769).

One unusual property of VP1 is that it often fails to react in immunoprecipitations or in immunoblots with many hyperimmune antisera made to purified virus particles (88) or with serum from naturally or experimentally infected children or animals (61, 69, 240). Other serum samples can precipitate VP1 (324; J. Cohen, unpublished observation), and the successful production of a VP1-reactive antiserum (by immunoprecipitation, immunofluorescence, and immunoblots) by immunizing animals with VP1 synthesized with a baculovirus expression system indicates that this protein is antigenic and immunogenic (66a). The different capacity of sera to react with VP1 after natural infection or parenteral immunization may be explained by the degree to which VP1 is accessible to the immune system. VP1 was partially accessible to iodination in single-shelled but not double-shelled particles (236). This suggests that either VP1 is exposed on single-shelled particles or the channels that penetrate into the inner core of particles are large enough for iodination reactions to occur inside the particles. Examination of whether antibody to VP1 reacts with single-shelled particles may help to answer this question.

VP2. VP2, encoded by genome segment 2, is the most abundant structural protein found in core particles (25, 194) and is the third most abundant protein in double-shelled particles. Lactoperoxidase-catalyzed iodination experiments suggest that VP2 is partially exposed on single-shelled particles (236), and this idea is supported by the demonstration that antibodies to VP2 are able to react by ELISA with single-shelled particles bound to plates (331). However, since direct binding of particles to plates can induce conformational changes in particles (45, 121), immunoelectron microscopic examination of whether these antibodies directly bind to particles would help to interpret these results. VP2 is highly immunogenic, and serum antibodies to this protein are a good indicator of prior infection (69, 324).

VP2 is the only structural protein shown to possess nucleic acid (dsRNA, ssRNA, and dsDNA)-binding activity when evaluated by an RNA overlay-protein blot assay (37).

TABLE 4. Rotavirus genome RNA segments and protein products[a]

Segment	Number of noncoding sequences[b] 5′	3′	Protein product[c]	Mol wt of nascent polypeptide[d] (no. of amino acids)	Mature protein modified (no. of amino acids)	Approx. %[e] (by wt) of virion protein	Temperature sensitive mutant group[f]	Remarks[g]
1	18	17	VP1	124,847 (1088)		2	C	Inner-core protein, slightly basic
2	16	28	VP2	102,431 (880)	Myristilated	15	F	Inner-core protein, RNA binding; leucine zipper (aa 536 to 559 and 665 to 686)
3	49	34	VP3	98,120 (835)		0.5	B	Inner-core protein, basic
4	9	22	VP4	86,782 (776)	Cleaved VP5* (529)[h] VP8* (247)[h]	1.5	A	Surface protein, hemagglutinin, protease-enhanced infectivity, neutralization antigen, virulence, putative fusion region (aa 384 to 401)
5	32	73	NS53	58,654 (491)			NA	Slightly basic, zinc fingers (aa 54 to 66 and 314 to 327)
6	23	139	VP6	44,816 (397)	Myristilated	51	G	Inner capsid protein, trimer, hydrophobic subgroup antigen, required for transcription
7	25	131	NS34	34,600 (315)			NA	Slightly acidic, RNA binding
8	46	59	NS35	36,633 (317)			E	Basic, role in RNA replication?
9	48 135	33 33	VP7(1) VP7(2)	37,368 (326) 33,919 (297)	Cleaved signal sequence, high-mannose glycosylation and trimming[i]	30	NA	RER integral membrane glycoprotein, cell attachment protein, neutralization antigen, two hydrophobic NH_2-terminal regions, bicistronic gene?, putative Ca^{2+}-binding site (aa 127 to 157)
10	41	182	NS20	20,290 (175)	NS29 → NS28 uncleaved signal sequence, high-mannose glycosylation and trimming		NA	Nonstructural RER transmembrane glycoprotein, two hydrophobic NH_2-terminal regions, role in morphogenesis, putative Ca^{2+}-binding site
11	21	49	NS26	21,725 (198)	NS28, phosphorylated, possible second type of modification		NA	Nonstructural, slightly basic; serine and threonine rich

[a] For A/Si/SA11 strain except genes 1, 2, and 5 for an A/Bo/Rf strain; see Table 3 for references.
[b] Number of 5′ noncoding sequences is up to the first AUG; number of 3′ noncoding sequences does not include the termination codon.
[c] Determined by biochemical and genetic approaches (see text). The size (in thousands) of the primary translation product is given for the nonstructural (NS) proteins.
[d] Molecular weights are calculated from the deduced amino acid sequences from nucleotide sequence data. The molecular weights are calculated from the largest potential open reading frame.
[e] Adapted from Liu et al. (194). These estimates are not totally consistent with estimates of the numbers of molecules of VP4, VP6, and VP7 (120, 780, and 780 molecules, respectively) made from structural analyses of particles (264; Prasad et al., submitted for publication).
[f] From Gombold and Ramig (123, 124). NA indicates that none was assigned.
[g] See text for references.
[h] There are two trypsin cleavage sites in SA11 4fM VP4 at amino acid 241 and 247. The indicated mature products are those based on the use of only the preferred second cleavage site (195, 196).
[i] Mature cleaved VP7 contains 276 amino acids.

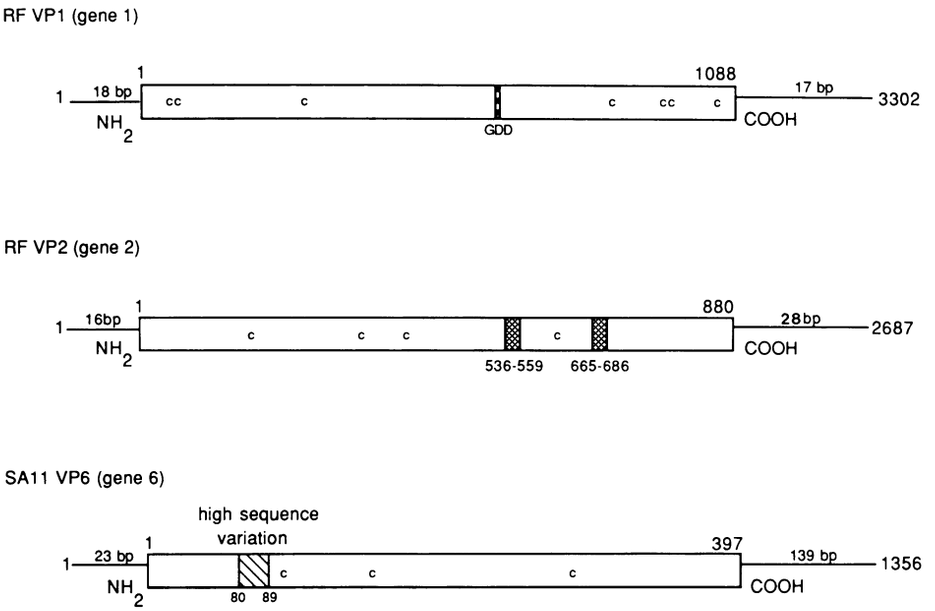


FIG. 3. Features of the core and inner capsid proteins VP1, VP2, and VP6. These schematics show features of each protein based on the amino acid sequence deduced from the nucleotide sequence. The total number of nucleotides in each gene, the length of the 5′ and 3′ noncoding regions, and the numbers of nucleotides or amino acids in each gene or deduced protein are shown. Cysteine residues, the polymerase consensus (GDD) signal in VP1 (aa 630 to 632), and the leucine zipper (aa 536 to 559 and 665 to 686) in VP2 are also indicated. The box (▧) shows a region of high sequence variation. Compiled from sequences of the genes which code for these proteins (see Table 3 for references).

VP2 binds ssRNA in preference to dsRNA, but the binding is not sequence specific. Analysis of the sequence of VP2 shows that this protein contains leucines at every seventh residue, starting at amino acids 536 and 665. Such a periodic array of leucines (leucine zipper [192]) is hypothesized to represent a part of a protein that molds it to interact with a target site on DNA (and probably on dsRNA) (Fig. 3) (188). VP2 may be a nucleocapsid protein that is bound tightly to the RNA segments. The apparent molecular weight (of 94,000) of VP2 calculated from its mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis differs from the molecular weight (of 102,431) deduced from the nucleotide sequence. This may result from the high percentage of predicted alpha helix in this protein. VP2 has been found to be myristilated, and although the functional significance of this is unknown, such modification in other viral systems has been found on scaffolding proteins important for the formation of virus particles (59). Epitopes on VP2 that cosegregate with subgroup specificity of VP6 also have been reported (331), but other biologic properties of VP2 are not yet known. MAbs to VP2 should be useful to further probe the function of this protein in the assembly of rotavirus particles or in RNA replication.

VP3. VP3, encoded by genome segment 3, is a minor structural protein that may comigrate with the outer capsid protein VP4 in many gel systems (194). In early studies, this gene product was reported to be a structural protein (307), a nonstructural protein (11), or a structural protein that was translated poorly in vitro and was synthesized and processed rapidly (217). These early studies all approached analysis of the genome segment 3 protein by comparing the proteins synthesized in infected cells with those translated in cell-free systems. A reevaluation of this question for SA11 involving the use of reassortant viruses and new methods of electrophoresis of the proteins on polyacrylamide gels confirmed that four proteins are located in single-shelled particles and three proteins are located in core particles (194), as shown earlier by others (25, 307). The poor cell-free translation of this gene product has been confirmed and remains unexplained. The sequence of gene 3 indicates that this segment codes for a protein of 835 amino acids with a predicted molecular weight of 98,120 (M. Liu and M. K. Estes, Nucleic Acids Res., in press). Analysis of the deduced amino acid sequence indicates that this is a basic protein that contains multiple repeats of amino acids. Homology with other RNA polymerases from other viruses also suggests that this protein is involved in RNA replication (Liu and Estes, in press).

VP6. VP6 is encoded by genome segment 6 and is the major structural protein in virus particles located on the outer surface of single-shelled particles. Biochemical characterization of VP6 removed from particles revealed that it is a trimer (129, 288), and three-dimensional structural studies of single-shelled particles have shown trimers to be present on the surface of these particles (264). Trimerization and formation of tubules is an intrinsic property of this protein, since VP6 synthesized in the absence of other viral proteins forms such structures (94). VP6 removed from particles will also form tubules and viruslike particles at low pH, but whether other proteins play a role in these reactions remains unclear (273). These results and biochemical data showing that VP6 is removed from inner-core particles prepared by treating single-shelled particles with chaotropic agents (25, 194, 293) counter the suggestion (based on iodination results with single-shelled particles) that VP6 is more internal than VP2 in particles (236). Alternative interpretations of these iodination results are that (i) the tyrosine residues required for iodination are not accessible in trimeric VP6; (ii) the channels leading into the inner particle are large enough to permit the iodination reactants (lactoperoxidase) to penetrate the particles and therefore label VP2; and (iii) VP2, which is a core protein that may bind to the RNA segments,

lines the channels and protrudes out of single-shelled particles.

Biochemical and immunologic approaches have been used to examine whether VP6 performs specific biologic functions during virus replication. Removal of VP6 from single-shelled particles results in a loss of transcriptase activity of particles. Readdition of VP6 to stripped single-shelled particles restores transcriptional activity, and addition of excess VP6 to stripped single-shelled particles inhibits transcription (25, 293). These experiments indicate that VP6 is required for polymerase activity, but they do not directly prove that VP6 itself is involved in transcription. VP6 may merely be important as a structural component of particles to maintain the proper conformation or organization of the viral core structure or transcriptional complex composed of one or more of the core proteins. VP6 is myristilated, and this modification may be important for formation of virus particles or for targeting single-shelled particles to the ER membrane for budding (59) (see below).

VP6 is both highly immunogenic and antigenic, and it is the most frequently targeted protein in diagnostic assays to detect virus particles. VP6 contains common (cross-reactive) epitopes shared by other group A viruses, and it can contain zero, one, or several forms of an antigen called the subgroup antigen (137, 168, 174, 190, 323). Whether VP6 plays a role in inducing protective immunity remains unclear.

Some MAbs to VP6 have been reported to possess low levels of activity that neutralizes virus infectivity when assayed in vitro (288). In addition, a synthetic peptide corresponding to aa 40 to 60 of VP6 has been reported to induce protective immunity in a mouse model of passive protection (111). These results contrast with most studies, which have failed to detect neutralization with anti-VP6 MAbs (119, 139, 150, 282, 305, 312) or with polyclonal monospecific serum raised to VP6 synthesized with a baculovirus expression system (94). It is possible that neutralization activity observed with VP6 antiserum is not specific but is caused by nonspecific trapping of infectious double-shelled particles owing to the aggregation of single-shelled particles present in most virus preparations. Neutralization activity detected with polyclonal monospecific serum made to gel-purified VP6 (178, 287) is difficult to interpret because of questions of the specificity of these sera. Contamination of the VP6 from gels with small amounts of the closely migrating VP7 can result in a neutralizing antiserum as a result of antibodies to VP7 (M. K. Estes, unpublished data).

The subgroup epitopes on VP6 detected by MAbs (137) have been used as an epidemiologic tool to monitor the antigenic properties of different virus strains. To date, attempts to map the location of the subgroup epitopes by using biochemical or molecular techniques have met with limited success because many of these epitopes are conformational and appear to be located on the trimeric but not monomeric structures (128). Analysis of VP6 with a panel of MAbs has indicated that VP6 possesses at least five nonoverlapping epitopes (261). Unfortunately, none of these MAbs or others to VP6 (128, 189, 190) have been directly compared with the original subgrouping MAbs to permit comparisons of results. Use of a rapid and simple method to map epitopes will facilitate such needed comparisons (45).

A rotavirus strain that was isolated from horses (FI-14) and that possesses both subgroup I and II epitopes has been identified (150). Sequence analysis of gene 6 of this virus suggested that two VP6 proteins may be produced from two different initiation codons (128). This study also determined the sequence of VP6 from several new virus strains and confirmed regions of sequence divergence recognized previously (36, 68, 99, 148); unfortunately, it did not shed further light on the map sites of the subgroup or common epitopes on VP6 (128). Comparison of the sequences of different virus strains has identified one region of fairly high sequence variation (Fig. 3), but no function has yet been mapped to this or other sites. Additional virus strains which lack subgroup I and II epitopes have been described (323), and sequence analyses of these strains may help define regions on VP6 which react with the subgroup MAbs. Use of MAbs which react with either the monomeric or trimeric form of VP6 should help us to understand the process of trimerization and the role of VP6 in the viral morphogenetic process (14; L. Svensson, personal communication).

Outer Capsid Proteins

VP4. VP4 is the protein product of genome segment 4, and it is a nonglycosylated outer capsid protein and a hemagglutinin in many virus strains (167, 208). It is responsible for a number of biologically important functions. In the presence of trypsin, VP4 is cleaved into VP5* (molecular weight approximately 60,000) and VP8* (molecular weight approximately 28,000), and this cleavage results in enhancement of viral infectivity (91, 97). Cleavage of VP4 has been shown to enhance penetration (but not binding) of the virus into cells (64, 113, 169). VP4 is also associated with restriction of the growth of certain rotavirus strains in tissue culture cells (137) and in mice (238) and with protease-enhanced plaque formation (167). Antibodies directed at VP4 neutralize rotavirus in vitro (45, 71, 137, 139a, 153, 179, 329) and passively protect mice against heterologous rotavirus challenge in vivo (210, 238). Further studies have suggested that VP4 induces protective immunity in animals (239) and is immunogenic in children and animals (69, 301, 324).

These diverse properties of VP4 highlight the importance of this protein in the biology of the rotaviruses. Figures 4 and 5 show features of VP4 based on recent sequence data (Table 3) and mapping of antigenic sites by sequencing escape mutants selected with MAbs that possess neutralization activity (204, 327). Direct amino acid sequence analysis of VP4, VP5*, and VP8* of a strain called SA11 4fM determined the site of trypsin cleavage (196). Both VP4 and VP8* had blocked NH_2 termini, and the VP5* of SA11 4fM was found to be composed of two polypeptides with slightly different amino acid sequences at their NH_2 termini. Comparison of these data with the deduced amino acid sequence of SA11 4fM VP4 identified two trypsin cleavage sites (arginine 241 and arginine 247), with the latter position being the preferred cleavage site (196). Subsequent nucleotide sequencing across this region of gene 4 in other strains revealed that the two trypsin cleavage sites at arginines are conserved in every rotavirus VP4 sequence analyzed (Fig. 5) (197). Further sequencing has also shown that the SA11 4fM gene 4 sequence is identical to gene 4 from bovine viruses and different from SA11 gene 4. The SA11 4fM virus apparently is a reassortant containing an SA11 gene 9 and a bovine gene 4 (Table 2) (171, 235).

In animal rotavirus strains, VP4 contains 776 aa. In human rotavirus strains, the deduced VP4 is 775 instead of 776 aa (126, 170), with the difference being that the human strains have lost one amino acid at residue 136 of the animal strains (Fig. 5) (170); VP8* of the human strains is, therefore, one amino acid shorter.

In several (but not all) human rotavirus strains, an additional potential trypsin cleavage site at either lysine or

arginine is found just before the 3′-proximal arginine site (aa 246 in human strains, aa 247 in animal strains [126, 196]). It is unknown whether these sequence differences at the trypsin cleavage site affect the biologic properties of different virus strains, but the additional trypsin cleavage site in some human viruses has been suggested to be correlated with virus virulence, because viruses with this extra site were originally isolated from children with symptomatic infections (Fig. 5, lines RV-5, WA, P, and VA70) (126, 130). Ninety-one amino acids on VP4 are conserved among strains from children with asymptomatic infections (Fig. 5, lines M37 and ST3), whereas a different amino acid is conserved at each of these sites among virulent rotaviruses (126). In addition, three of these amino acids are located within the 6-aa connecting peptide between the two cleavage products of VP4 (126). Some or all of the 91 differences in VP4 amino acids between viruses from asymptomatic and symptomatic children have been postulated to be responsible for the difference in virulence of these two groups of human rotaviruses, because none of the other 10 rotavirus gene segments are conserved among asymptomatic or virulent strains (108). The significance of these findings is unclear, because far fewer amino acids are conserved if one compares the conserved sequences in asymptomatic strains with those in virulent animal strains (Fig. 5) and there is no proof that the strains from asymptomatic newborns are really avirulent. Other studies have suggested that host (rather than viral) factors may cause asymptomatic disease in newborns, because virus strains that cause disease in older children often cause asymptomatic infections in newborns (338, 344).

The mechanism of cleavage activation of infectivity is not clear from the sequence analyses alone. It is thought that cleavage of VP4 activates an early step of replication which may be triggered by one or both of the terminal regions generated by the cleavage or by a possible conformational change in cleaved VP4 molecules. Trypsin activation of orthomyxoviruses and paramyxoviruses generates a highly conserved apolar amino terminus, and the infectivity of these activated viruses can be blocked by oligopeptides that mimic this sequence (274, 275). The new terminus generated by cleavage in the rotavirus VP4 is not hydrophobic; instead, it contains many polar amino acids (some of which might be charged at neutral pH). Therefore, the mechanism of activation of rotavirus infectivity is apparently different from that postulated for other viruses. Since rotavirus infectivity has been shown to be enhanced by trypsin, elastase, and an uncharacterized acid-stable enzyme in pancreatin (but not by chymotrypsin [132]), further examination of the sites of natural cleavage may be useful to understand activation. It also remains unknown whether cleavage at both sites results in removal of the small intervening peptide and whether the minor and major cleavage sites shown to be used for SA11 4fM are used identically for other virus strains.

Studies are just beginning to determine whether the cleavage region (and the intervening peptide) possesses any functions. The region (aa 228 to 241) adjacent to the cleavage region has been shown to induce neutralizing antibodies by immunization of rabbits with a synthetic peptide to this region. The antipeptide serum showed strain-specific low-level neutralizing activity, and it bound to virus particles in ELISAs and aggregated particles by immunoelectron microscopy (317). Neutralization titers were increased (from 800 to 3,200) following assay of trypsin-treated virus-antipeptide serum complexes. Trypsin treatment of virus particles also has been shown to result in loss of reactivity of the virus with a nonneutralizing antibody to the glycoprotein VP7 (72). Knowing the location of this epitope might help in mapping possible sites of interaction between VP4 and VP7.

A region in VP4 (aa 384 to 401) has been identified that shares some similarity with an internal fusogenic hydrophobic domain thought to be located about 80 aa from the N terminus of the E1 glycoprotein of Sindbis and Semliki Forest viruses (118, 204). These similarities with the alphavirus E1 protein are of interest, and it is tempting to speculate that this region in VP4 is one which acts alone (or in cooperation with other hydrophobic regions of VP4 [as in E1]) to induce membrane fusion. This is an attractive hypothesis because cleavage of VP4 has been associated with internalization of virus (perhaps by mediating direct membrane penetration) into cells (64, 113, 169) and acidification of endosomes is not important for virus entry (114, 176, 201). Conservation of the sequences in this putative fusion region of VP4 of different rotavirus strains lends credence to the suggestion that it plays an important function in the replication cycle. However, it should be noted that the exact location of this fusogenic domain in E1 is not proven, since experiments with MAbs have shown that fusion activities are located at different sites in the E1 molecule (29, 50, 51, 245, 296). In addition, it remains unknown whether this putative fusion activity would act to mediate rotavirus entry into cells or to mediate virus maturation that involves budding across the membrane of the ER (see below), or both.

Analysis of the deduced amino acid sequence of VP4 indicates that the amino-terminal 70% of the protein is rather hydrophobic. The predicted amino acid sequence of VP4 has a net negative charge at pH 7.0, and secondary-structure predictions suggest that the amino terminus (prior to the trypsin cleavage site) contains numerous random coils and turns, suggesting a globular structure. Four cysteines in VP4 (aa 216, 318, 380, and 774) are conserved in all the rotavirus strains sequenced to date (126, 170, 171, 204, 235). An additional cysteine is present at aa 266 in the human M37 strain (126) and at aa 203 in SA11, SA11 4fM, bovine 486, and RRV. It has been proposed that the trypsin cleavage site is kept accessible through disulfide bonds at cysteines 203 and 216 in VP8* and at cysteines 318 and 380 in VP5*; lack of Cys-203 was suggested to explain the poor growth of human rotavirus strains in cell culture (204). This seems unlikely since the bovine UK virus, which grows well in vitro, has a serine at aa 203 (171). However, the regions flanking the trypsin cleavage sites (aa 224 to 235 and 257 to 271) are relatively highly conserved in all strains, and these could serve to hold the cleavage sites in the proper conformation for cleavage.

Sequencing of escape mutants selected with neutralizing MAbs has identified amino acids involved in homologous and heterologous neutralization (204, 327). VP8* contains predominantly type-specific sites, and five such sites (Fig. 4, sites 1 to 5) have been localized in VP8* (204, 304). Cross-reactive MAbs from two different laboratories have been located on VP5* clustered in a relatively limited area at aa 306, 388, 393, 434, and 440 (204, 327). These positions are based on the numbering for the animal rotavirus strains (i.e., aa 306 would be 305 in the human virus VP4). One MAb (site 6) that neutralizes viruses in serotypes 3, 5, and 6 (based on VP7 reactivities) is located at aa 393 in the middle of the conserved hydrophobic region and the putative fusion region. Other cross-reactive MAbs (epitope II) for human viruses cause changes at aa 392 and 439 (393 and 440 of animal strains), suggesting that this site is conformational (327). Another cross-reactive site (epitope I) is sequential (or

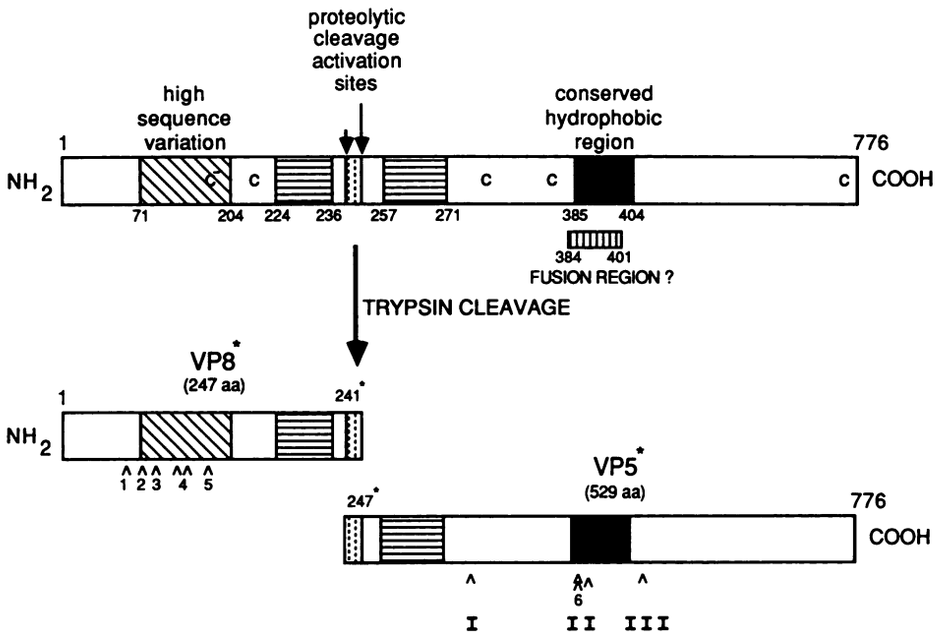


FIG. 4. Features of the outer capsid protein VP4. Schematic of structural and antigenic properties of VP4 based on the analyses of nucleotide sequences of different virus strains and escape mutants selected with neutralizing MAbs. Symbols: ▤, regions of sequence conservation among different virus strains; ▧, region of greatest sequence variation in VP8*; ▥, potential fusion region; ⇊, sites of cleavage by trypsin. Conserved cysteines (C) at aa 215, 317, 379, and 773 and the cysteine (C^-) at aa 203 not present in some human rotavirus strains are shown. The locations of neutralization epitopes defined by Mackow et al. (204) are shown by ^ and a number from 1 to 6; the epitopes defined by Taniguchi et al. (327) are indicated by ^ and a Roman numeral (I to III). The peptide possibly removed by trypsin cleavage at aa 241 and 247 is shown (▯). The numbering shown here for VP4 is based on a protein of 776 aa as determined for SA11 4fM, RRV, and Bo/486. VP4 of human rotavirus strains contains 775 aa, lacking an amino acid at residue 136 (see Fig. 5). See text for references.

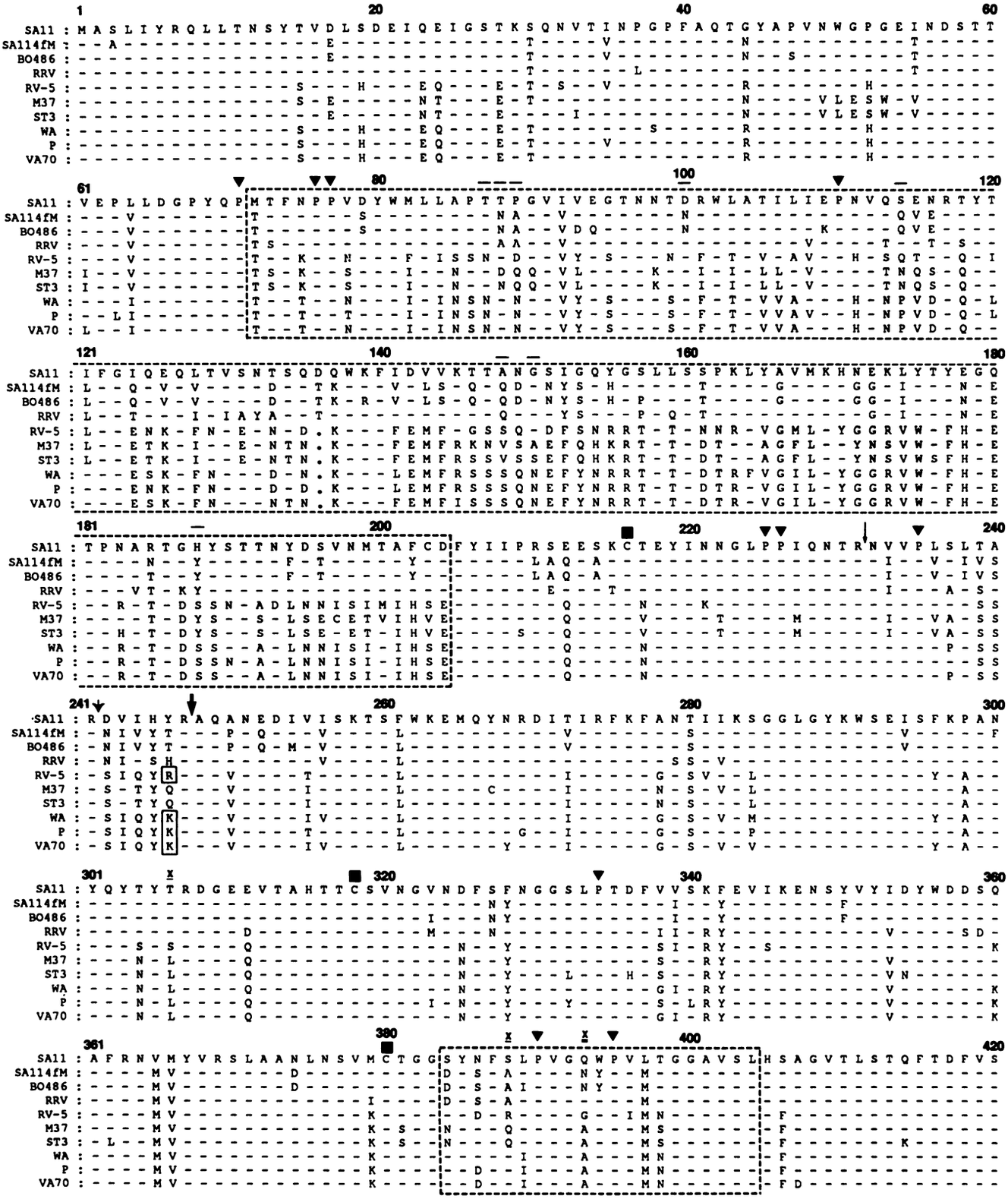
linear) on the basis of reactivity with a synthetic peptide from aa 296 to 313, and aa 305 of the human VP4 is apparently critical for this epitope (327). The cross-reactive epitope I site and an epitope III site (at aa 433) have been identified only by one set of MAbs (327, 328). None of these MAb-identified neutralization regions corresponds to the cleavage region shown to be immunogenic by injection of synthetic peptides from the cleavage site (317) (see above). This suggests that variability exists in the immunogenicity of distinct regions of VP4, and the relative immunogenicity of these epitopes remains to be clarified.

Comparisons of the segment 4 sequences of viruses in different serotypes to look for regions of diversity show that homologies are not evenly distributed across VP4 (Fig. 5). The most distinct sequence variation is seen between aa 71 and 204 in VP8* (Fig. 5). It is tempting to speculate that VP8* is externally exposed on the virus particle, since VP8* contains the locations of greatest sequence diversity and of binding of the strain-specific MAbs (whereas cross-reactive sites have been mapped to VP5*) and since VP8* is predicted to be globular. If readily exposed, the sequence diversity in VP8* could easily arise by immune selection. No function or neutralization epitopes have yet been mapped to this variable domain. A second region of sequence variations is found between aa 578 and 608.

Attempts to locate other functional domains on VP4 have not yet been successful. Genetic analyses show that VP4 is the hemagglutinin, and hemagglutination activity has been demonstrated with VP4 molecules expressed by baculovirus recombinants (202; S. E. Crawford and M. K. Estes, unpublished data). The site on VP4 that binds to erythrocytes remains unknown, but it may involve several regions of VP4 because MAbs to both VP5* and VP8* can inhibit hemagglutination (45, 139a, 179, 304). However, some of these MAbs may inhibit hemagglutination by steric hindrance. Site-specific mutagenesis and high-resolution structural studies of particles or of individual proteins are needed to answer these questions.

VP7. VP7 is the second most abundant capsid protein and is encoded by genome segment 9 of SA11, genome segment 8 of the UK bovine virus, and genome segment 7 of rhesus rotavirus. This outer capsid glycoprotein is highly immunogenic and induces neutralizing antibodies. Recognition that this neutralization antigen is a structural glycoprotein stimulated numerous studies of the structure, biosynthesis, and functions of VP7. Biochemical analyses rapidly determined that (i) VP7 is a glycoprotein that contains only N-linked high-mannose oligosaccharide residues, which are added cotranslationally as this protein is inserted into the membrane of the ER, and (ii) VP7 is an integral membrane protein with a luminal orientation whose oligosaccharides are modified by trimming which occurs only in the ER (89, 165; see the section on eucaryotic protein targeting and oligosaccharide processing).

The nucleotide sequence of VP7 from virus strains in six human serotypes and two animal serotypes and the deduced protein amino acid sequence reveal several interesting and possibly biologically relevant features of this gene and its deduced protein (Fig. 6 and 7). The nucleotide sequence predicts an open reading frame of 326 amino acids beginning with an initiation codon with a weak consensus sequence (186). A second, in-frame initiation codon with a strong consensus sequence lies 30 codons downstream. Each of these first two initiation codons precedes a region of hydrophobic amino acids (H1 and H2) which could act as a signal sequence to direct VP7 to the ER. A third in-frame initiation


SA11
SA114fM
BO486
RRV
RV-5
M37
ST3
WA
P
VA70

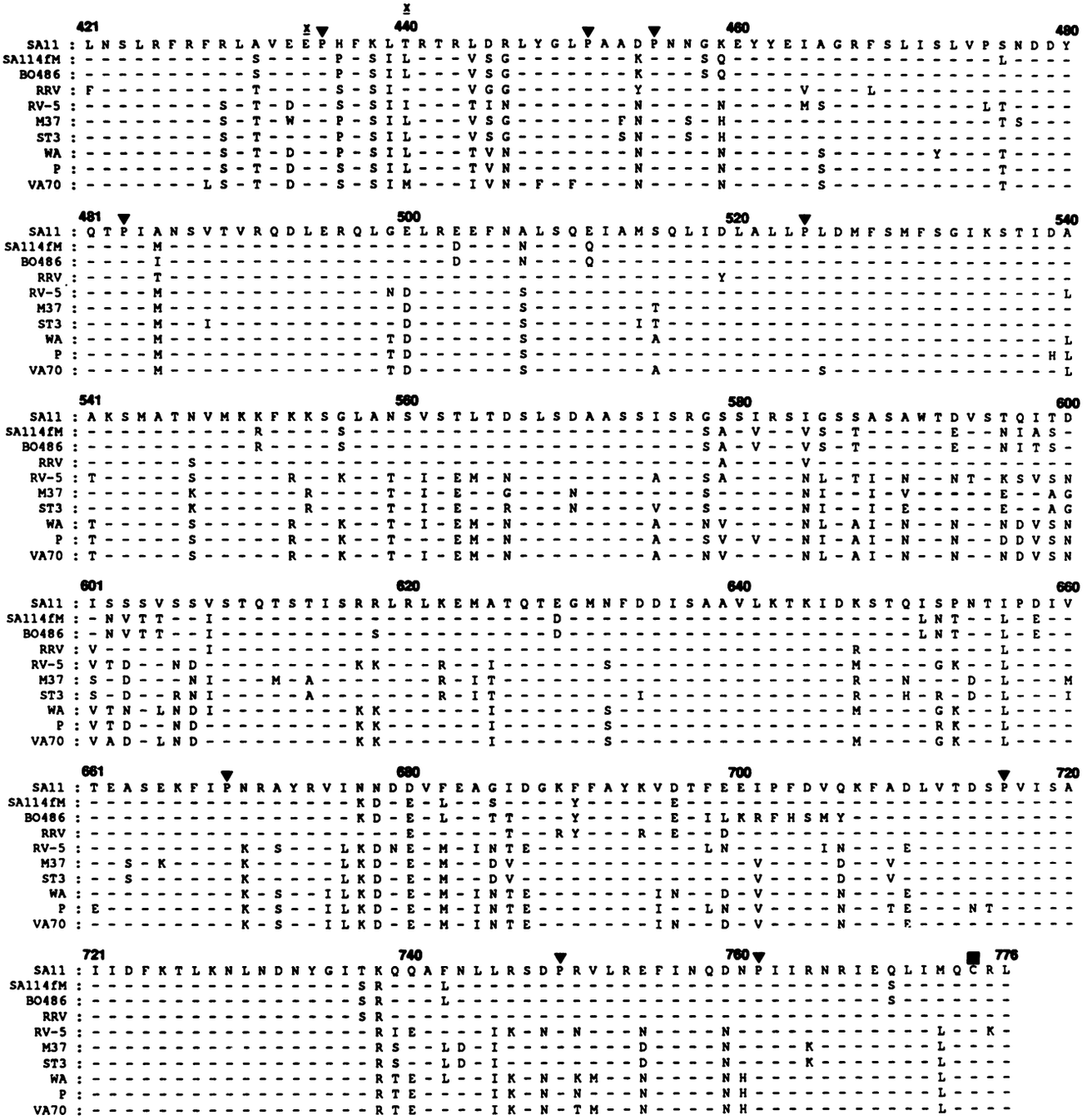


FIG. 5. Comparisons of the deduced amino acid sequences of VP4. The sequences of six strains of human rotaviruses and four strains of animal rotaviruses are shown with cysteine (■) and proline (▼) residues conserved in all strains being indicated. The arrows highlight the trypsin cleavage site(s) known to be used in SA11 at aa 241 and 247 and another potential site at aa 231. The additional potential trypsin cleavage site found in some human rotavirus strains is boxed, and dots (●) show the amino acid missing in human strains at residue 136 of the animal strains. The region of high sequence variation in VP8* (aa 71 to 204) and the relatively conserved hydrophobic region in VP5* (aa 385 to 404) which contains the putative fusion region are highlighted by dashed boxes. The sites of amino acid changes of neutralization escape mutants are marked by a bar. Altered sites found in neutralization escape mutants with MAbs that react with viruses in more than one serotype are marked by an X; X indicates site of amino acid changes of neutralization escape mutants, as well as an altered site that reacts with viruses in more than one serotype. The sequences shown are for SA11 (235), SA11 4fM (195, 196), bovine 486 (262), rhesus RRV M18006 (204), human RV-5 (170), and human M37, ST3, WA, P, and VA70 (126). The human viruses RV-5, WA, P, and VA70 were isolated from children with symptomatic infections; the human viruses M37 and ST3 were isolated from children with asymptomatic infections (126). The similarity of the SA11 4fM to the bovine 486 protein is striking. The sequence of the SA11 gene 4 from other laboratories is distinct (171, 235; Estes and Crawford, unpublished), suggesting that gene 4 of SA11 4fM is a bovine gene derived by reassortment (see text). Only amino acids which differ from the sequence of SA11 are shown.

codon, found 42 nucleotides downstream from the second hydrophobic domain, is not present in all strains. The simian rotavirus SA11 strain contains one potential N-linked glycosylation site at aa 69 which is utilized; this site is found in all other viruses, including the bovine UK strain, but it is missing in the bovine NCDV and RF strains. These bovine and other strains contain other potential glycosylation sites at aa 146, 238, and 318 (54, 122, 135, 142), confirming biochemical studies indicating that some strains contain two glycosylation sites in VP7 (183, 294). The location of the second site of glycosylation is not known for any strain. A region (aa 133 to 146) that is well conserved in rotavirus strains examined so far (except ST3) shares similarities with Ca^{2+}-binding sites of other proteins. This region is between two predicted alpha helices and contains six amino acids with oxygen atoms on their side chains that may coordinate the interaction with calcium. The sequence divergence in this putative region for VP7 of the human ST3 strain suggests that differences in the calcium-binding capacity of distinct strains may exist. The presence of this Ca^{2+}-binding site may explain why VP7 can be removed from double-shelled particles by chelation of calcium ions (67). Although no direct proof of calcium binding to VP7 has yet been shown, calcium has been shown to bind to double-shelled and not to single-shelled particles (299).

The biosynthesis of VP7 from SA11 has been studied in some detail. Because the two in-frame initiation codons are conserved in all rotavirus strains examined to date, this gene is potentially bicistronic. Furthermore, since translation from such multiple in-phase initiation codons has biologic significance in other viral systems (186, 234, 251), it is of interest to know whether the second in-phase initiation codon is used. Two bands of VP7 are detectable when the proteins in preparations of purified SA11 are analyzed on polyacrylamide gels (49, 316), and similar bands also were noted in infected cells by others (82). On the basis of biochemical studies and cell-free translation of VP7 in wheat germ lysates, it was suggested that these bands may result from the initiation of translation of two primary gene products from the first two in-phase initiation codons, with subsequent distinctive processing of these proteins (49). These studies also hypothesized that the final protein products would have different amino termini owing to different processing of signal peptides of the two primary products (49).

Studies designed to determine the site of cleavage of the signal peptide in VP7 initially were unsuccessful because viral VP7 has a blocked amino terminus (10, 316). However, reevaluation of this question by using gene constructs containing altered initiation codons showed that proteins translated from either initiation codon are processed in vitro in rabbit reticulocyte lysates to yield products identical in size (316). Therefore, the primary translation products appear to be cleaved at the same site. Amino-terminal sequence analysis identified Gln-51 as the amino-terminal residue of at least some molecules of VP7 from purified virus. Gln-51 is conserved in all strains, and pyroglutamic acid has been identified as the blocking group (316). Since these studies indicate that the processed products translated from either the first or second AUG are the same, it would seem that the first AUG is not needed to produce VP7. This raises the question of why this first initiation codon is conserved in all VP7 genes. Possibly this codon is used in vivo to produce low levels of a protein with a subtly different property or the conserved RNA sequences in that region have a specific function. Further studies are required to resolve this issue.

Experiments with bovine rotavirus and human rotaviruses indicate that VP7 is the cell attachment protein (113, 213, 287). Initial studies showed that a polyclonal monospecific antibody, made to native VP7 purified by isoelectric focusing in glycerol gradients, blocked the adsorption of radiolabeled virus to MA104 cells (213). Later studies reported that soluble VP7 in infected cells binds to monolayers of intact target cells. Binding of soluble VP7 is blocked by virus particles, and binding of radiolabeled virus is blocked by anti-VP7 (but not anti-VP4) MAbs (113, 287). Adsorption of rotavirus to tissue culture cells has also been shown to be independent of trypsin treatment of virions normally needed for virus infectivity (64, 113).

In studies to identify and characterize the domain on VP7 responsible for binding to cells, a 14,000-molecular-weight fragment of VP7 (the 14K fragment) and a peptide containing aa 275 to 295 blocked adsorption of the virus to cells. MAbs to this peptide also blocked adsorption (111, 287). The 14K fragment of VP7 was reported to be an immunodominant domain on VP7 which induces neutralizing antibodies (286). MAbs that reacted with the 14K fragment and the peptide containing aa 275 to 295 also reacted with other serotypes, suggesting that this region may be conserved among rotaviruses (111, 287). Glycosylation was not found to be essential for the reactivity of the 14K fragment with antibodies and with cell surface receptors; in contrast, disulfide bridging was essential for binding (287), although, surprisingly, the peptide that blocked binding did not contain any disulfide bonds (111). This peptide was also reported to induce protection against virus challenge in the murine model of passive protection (111).

This proposed neutralization, cell-binding, and protective domain on VP7 remains unconfirmed, and it is accepted with reservation because of the following seemingly conflicting results. Sequence analyses of different virus strains indicate that although this region is hydrophilic, it is not absolutely conserved as might be expected from the data summarized above (Fig. 7) (54, 135, 206). Other investigators also used the same VP7 peptide (aa 275 to 295) from SA11 virus to immunize rabbits (142). The SA11 peptide was immunogenic, and antiserum produced to it reacted with denatured VP7 on immunoblots, but this serum failed to bind to or neutralize virus (142). Finally, others have reported that the soluble protein in infected-cell lysates that adheres to cells is NS35, not VP7 (D. M. Bass, E. R. Mackow, and H. B. Greenberg, Gastroenterology **96**(5, part 2):A30, 1989). The reason for these very different results is presently unclear.

One possible explanation for the conflicting results (described above) and a major problem in evaluating the immunogenicity of peptides or expressed rotavirus proteins (see below) is the difficulty of obtaining seronegative animals for immunization. Rotaviruses are ubiquitous, and many investigators have shown that sera from "normal" animals (including prebleeds and negative controls) contain antibodies to rotavirus proteins (241, 286, 318). Therefore, all animals must be carefully screened to prove that they are seronegative, and conclusions from studies of animals with preexisting antibodies must be interpreted carefully, because biologic activities in sera from primed and boosted animals may not accurately reflect the immunogenicity of the inoculum.

Conformation maintained by disulfide bonds has been shown to be important in maintaining neutralizing epitopes on VP7. This was first suggested by the inability to produce hyperimmune antiserum with neutralizing activity against VP7 purified from denaturing polyacrylamide gels (20, 95, 287). The inability of many neutralizing MAbs to react with

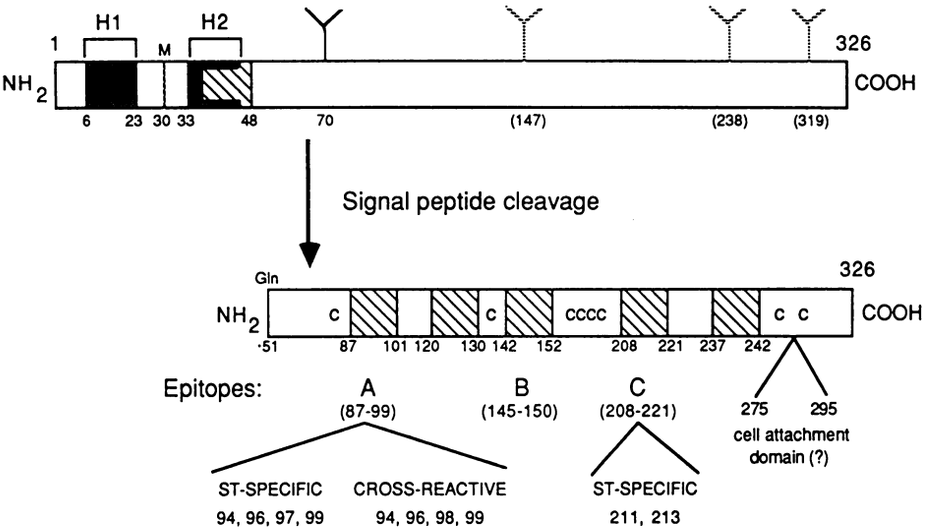


FIG. 6. Features of the outer capsid protein VP7. The figure shows a schematic of structural and antigenic properties of VP7 based on the analyses of nucleotide sequences of different virus strains and escape mutants selected with neutralizing MAbs. The location of the two amino-terminal conserved hydrophobic regions (■), the second in-phase methionine (M) at a preferred initiation codon (see text), the one glycosylation site known to be used in SA11 (Y), additional potential glycosylation sites found in other virus strains (Y_1), conserved cysteine (C) residues, and the one known site of signal peptide cleavage are shown. The lower diagram illustrates the regions of sequence variation (▧), the proposed cell attachment domain (aa 275 to 295), and the locations of amino acid changes in escape mutants selected following reactivity with serotype-specific and cross-reactive neutralizing MAbs. See text for references.

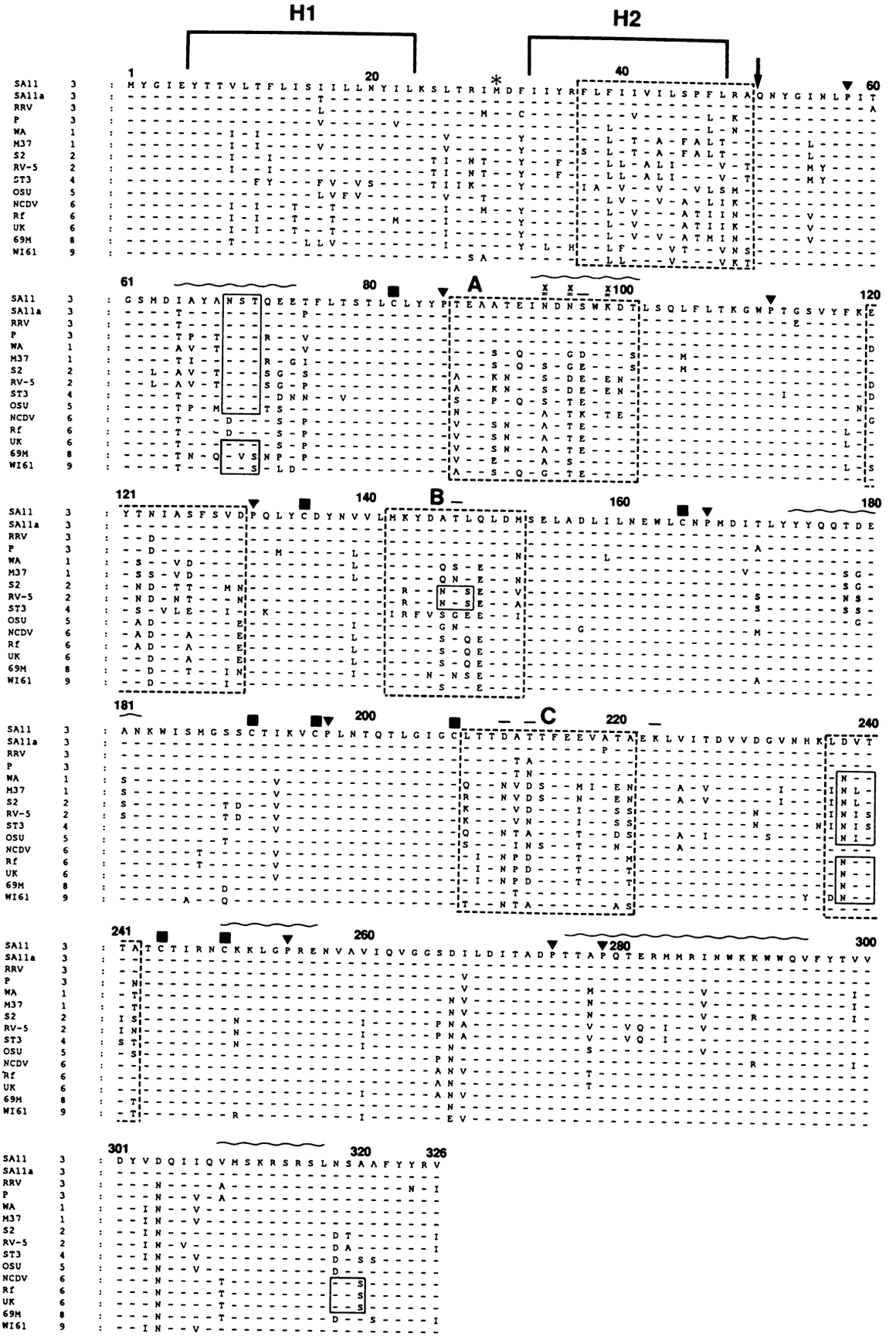
denatured VP7 in Western immunoblots or by immunoprecipitation (71, 121, 144, 329, 330) and the failure of linear synthetic peptides derived from different regions of VP7 to elicit neutralizing antibodies (142) confirm these conclusions.

Studies of escape mutants made with neutralizing MAbs and comparisons of the deduced amino acid sequences of VP7 of viruses with different serotypes have identified regions on this protein that are involved in neutralization (Fig. 6 and 7). Six regions are evident on the basis of the divergence of nucleic acid sequences between viruses with different serotypes (Fig. 7). However, only five of these are apparently present in mature VP7, and only three of these regions (A, B, and C), located at aa 87 to 101, 142 to 152, and 208 to 221, respectively, have been confirmed to be involved in neutralization, based on mapping the sequence changes in neutralization escape mutants (84, 203, 326). The mapping of such mutations (in escape mutants made with neutralizing MAbs that are strain specific or that cross-react between viruses in different serotypes) also has allowed locations of cross-reactive (in region A) and strain-specific (in regions A and C) epitopes to be determined (Fig. 7) (203, 326). These results, together with competition results of the neutralizing MAbs, indicate that the three-dimensional folding of the native protein brings regions A and C in close proximity (84). Region C appears to be the most important, since a mutant containing an amino acid substitution within this region was found to have a markedly increased resistance to hyperimmune antisera (84). On the basis of these results, region C has been suggested to be an immunodominant antigenic site.

The study of viruses with different numbers of glycosylation sites (from zero to two) on VP7 is beginning to show that glycosylation can modulate the biologic and antigenic properties of VP7. The observation that an SA11 variant with a nonglycosylated VP7 is infectious and possesses the biologic properties of hemagglutination and serotype specificity indicates that glycosylation is not essential for these functions (254; Estes, unpublished). Selection and characterization of neutralization escape mutants of this nonglycosylated variant of SA11 found that some mutants possessed an amino acid substitution at residue 238 of VP7, whereas mutants of wild-type SA11 (selected with the same antibody) contained an amino acid substitution at residue 211 (in the C antigenic region). In both cases, the mutations produced new potential glycosylation sites, and these were found to be utilized. These mutations led to gross antigenic changes, which were found to be reversible upon removal of the attached carbohydrate (47). Andrew et al. (6) also postulated that the presence of carbohydrate at aa 146 in serotype 2 viruses blocked cross-reactivity in immunoblots with an anti-SA11 VP7 antibody that reacted with viruses with serotypes 1, 3, and 4. Finally, the presence of a potential glycosylation site at aa 238 in the Saint Thomas type 4 VP7 (which is not present in the VA70 type 4 VP7) may explain why these two type 4 VP7 proteins can be differentiated by serologic assays and some MAbs (120, 135). These results show that carbohydrates can have an important role in influencing the exposure of antigenic determinants on VP7. However, this is not a universal phenomenon, since the addition of a new carbohydrate side chain at residue 99 in another neutralization escape mutant did not dramatically affect antibody binding to either the A or C region (203).

Identification of the location of specific glycosylation sites on VP7 in strains with several potential sites may help interpret existing and future studies, including anticipated high-resolution structural studies of VP7. It will be particularly interesting to see whether the potential glycosylation site at aa 318 is used, since this carboxy-terminal region of VP7 should be exposed, based on the reactivity of virus particles with an antiserum made to the carboxy-terminal 8 aa of SA11 VP7 (318). Since this serum also reacts with the human serotype 1 Wa virus and with a serotype 4 (Hochi) virus, this site is probably not used or the attached carbohydrates do not interfere with the immunoreactivity of the 7 aa closest to the carboxy terminal.

The effects of glycosylation on antigenicity are not sur-


H1
H2
A
B
C
SA11
SA11a
RRV
P
WA
M37
S2
RV-5
ST3
OSU
NCDV
Rf
UK
69M
WI61

prising, owing to earlier studies with influenza virus. For example, the presence of a new carbohydrate moiety was clearly shown to be essential in preventing a MAb from binding to a mutant virus, and antibody binding reappeared when carbohydrate attachment was inhibited by growing virus in the presence of the glycosylation inhibitor tunicamycin (306). The rotavirus results, however, differ from the influenza virus results, because with SA11 an effect on reactivity with polyclonal hyperimmune antibody (not merely MAb) was demonstrated, indicating that a major antigenic site is involved.

Other biologic differences between rotavirus strains (which may be due to differences in glycosylation) are worth mentioning. Although isolation of the infectious SA11 variant with a nonglycosylated VP7 indicates that the presence of oligosaccharides at the one site (Asn-69) in the SA11 VP7 is not essential for cell binding or virus infectivity, the presence of oligosaccharides at other sites in VP7 may have previously unrecognized effects. In the KUN human serotype 2 rotavirus, two VP7 proteins of molecular weight 37,000 and 35,000 (37K and 35K proteins) were detected in infected cells (294), and only the faster-migrating 35K band possessed cell-binding activity (113). Sequence analyses show that serotype 2 viruses possess additional potential glycosylation sites at residues 146 and 238 (135). Since the two VP7 bands in this strain reportedly reflect differences in glycosylation, the presence of oligosaccharide at a second site in the more slowly migrating 37K VP7 may prevent VP7 from binding to cells. If the cell attachment site (described above) at aa 275 to 295 is correct, and if glycosylation at a second distinct site (aa 146 or 238) can prevent binding to cells, one must propose that addition of oligosaccharide to this site alters the overall conformation of VP7. This remains speculative, but possible, on the basis of recent data showing the importance of conformation and oligosaccharide sites in antigenicity (see above). This observation suggests the interesting possibility that the two VP7 glycoproteins in the KUN virus strain are functionally distinct.

A significant reduction in outer-shell assembly has been reported when bovine and human rotavirus strains (which contain two sites of glycosylation of VP7) are grown in the presence of tunicamycin (285, 322). This contrasts with the observation that double-shelled SA11 is made under similar conditions (254). The outer capsid of human rotaviruses also is easily removed during purification (329). These results may indicate that assembly or stability of the outer capsid of viruses with more than one carbohydrate moiety may be more complex than for viruses which contain no glycosylation sites or only one glycosylation site in VP7. Continued studies with a variant of SA11 (which apparently possesses a very stable outer capsid and a VP4 with distinct properties) may help clarify how interactions between VP4 and VP7 molecules can influence the biologic and antigenic properties of both outer capsid proteins (44, 56).

Many MAbs and polyclonal antibodies to VP7 possess hemagglutination inhibition activity. This activity is thought to be due to steric hindrance because VP4, and not VP7, is the hemagglutinin. This also suggests that VP4 and VP7 interact closely, and it seems likely that such interactions will ultimately be shown to influence many more biologic properties of these viruses than are currently recognized.

Nonstructural Proteins

Our current knowledge of the functions of the nonstructural proteins is generally less extensive than that of the capsid proteins. However, sequence data have begun to yield some information on general protein structure and possible clues about function (Fig. 8). Ongoing studies of expressed nonstructural proteins are expected to quickly provide more specific information on the roles of these proteins in the replication cycle.

NS53. NS53 is a nonstructural protein encoded by SA11 genome segment 5. This protein has been found in infected cells but not in virus particles by most (11, 88, 149) but not all (217) investigators. The kinetics of polypeptide synthesis in rotavirus-infected cells show that NS53 is made and is detectable predominantly at early times after infection (88, 236). The synthesis of this protein is apparently controlled, because in infected cells the levels of NS53 produced are low relative to those of the other viral proteins, whereas in cell-free systems, NS53 is synthesized in large amounts relative to the other viral proteins (88, 208). However, nothing is known about the mechanism(s) that regulates the synthesis of this protein in infected cells. NS53 is a basic protein with a net positive charge of 9 at pH 7.0 on the basis of the deduced amino acid sequence (38).

More recent analyses show that NS53 contains predicted zinc-binding sites between aa 54 to 66 and 314 to 327. Despite extensive sequence diversity between simian and bovine genes, these predicted metal-binding sites are conserved (D. B. Mitchell and G. W. Both, personal communication). These sites also appear to be functional on the basis of preliminary direct binding of zinc (Fig. 8) (J. Cohen, M. Bremont, and M. K. Estes, unpublished data). Zinc binding is a characteristic of proteins that bind to nucleic acid (102, 295), but this was not demonstrated when the direct binding of radiolabeled nucleic acids to rotavirus proteins (immobilized on nitrocellulose filters) was evaluated (37). Small amounts of NS53 have been detected in complexes isolated from infected cells associated with replicase activity (246), and NS53 has been detected in protein complexes (in association with NS34 and RNA) that form following cell-free translation of mRNAs (K.-S. Au and M. K. Estes, unpublished data). However, it is unknown whether these complexes are associated with replicase activity. Further work to directly show whether NS53 is involved in RNA replication should be facilitated by the recent successful synthesis of this protein in *Escherichia coli* and with the baculovirus expression system (38; J. Cohen and M. K. Estes, unpublished data). Interestingly, NS53 is the first rotavirus protein to be successfully expressed in *E. coli* without problems of

FIG. 7. Comparisons of the deduced amino acid sequences of VP7. The sequences of eight strains of human and of animal rotaviruses are shown, and conserved cysteine (■) and proline (▼) residues are highlighted as in Fig. 5; conserved hydrophilic (⁓) regions are also shown. The two amino-terminal hydrophobic regions (H1 and H2), the methionine at the initiation codon used primarily in cells (*), the known site of cleavage of the signal peptide (arrow), the sites of potential glycosylation (boxes), the sites of sequence variation (dashed boxes), and the locations of known neutralization epitopes (A, B, and C) are also shown. The VP7 sequences of SA11 as reported by Both et al. (34) and Arias et al. (10) (called SA11a in the figure) are shown for comparison. Three changes from the original SA11 sequence by Both et al. (34), C-32 to F, L-37 to F, and T-65 to I, were reported for a separate clone studied by Stirzaker et al. (316). These are included in the SA11 sequence. Only amino acids which differ from the sequence of SA11 are shown.

degradation or toxicity necessitating the engineering of a fusion protein to express other proteins (9, 38, 219).

Genome segment 5 possesses other unusual properties. Nucleic acid hybridization studies indicate that the nucleotide sequence of this gene may be the least conserved (101, 297). In addition, genome segment 5 shows nonrandom reassortment when the distribution of individual segments which reassort in animals or in cells infected in vitro with two different rotavirus strains have been examined (4, 123). The basis for this is currently unknown, but it suggests that certain gene 5 segments may be more favorable for replication. In addition, gene 5 has often shown genome rearrangements (158). Taken together, these data suggest that NS53 may interact with genome segments during RNA replication or genome assortment.

A recent paper described an empty capsid-specific protein with a molecular weight of 55,000 (42). Although the origin of this protein was not identified, the possibility that it is NS53 and that it is present only in empty capsids is an intriguing one. Perhaps NS53 is involved in packaging genomic RNA segments. It will also be interesting to determine whether any of the unusual properties of this gene are related to its containing the complementary sequence of the 3′-terminal consensus sequence internally.

NS35. NS35 is encoded by genome segment 7, 8, or 9, depending on the virus strain. For example, SA11 genome segment 8, RRV genome segment 9, and bovine UK virus genome segment 7 each encode this protein (123, 208, 217). The deduced amino acid sequence of this protein indicates that it is basic, and immunocytochemistry experiments have localized this protein in association with viroplasms in infected cells (255). These data and the fact that SA11 group E temperature-sensitive mutants with mutations that map to this gene have an RNA-negative phenotype (267, 268) and produce a large proportion of empty particles (271) suggest that NS35 is involved in the replication of RNA or the packaging of ssRNA into subviral particles. The presence of a helix-turn-helix motif between residues 160 and 218 in this protein is consistent with possible interactions with nucleic acid. It will be of interest to determine whether these sequences are altered in the group E temperature-sensitive mutants. NS35 has been detected in subviral particles isolated from infected cells and characterized to possess replicase activity (145). However, NS35 denatured and immobilized on nitrocellulose membranes did not bind to radiolabeled nucleic acids (37). Characterization of the RNA-binding and replicase activity of NS35 produced by using expression systems will help define these and other possible functions of NS35. One MAb for this protein has been identified (255; H. B. Greenberg, unpublished data). A report suggesting that this protein (and not VP7) is the cellular attachment protein raises the question of whether this is really a nonstructural protein (Bass et al., Gastroenterology **96**(5, part 2): A30, 1989).

NS34. NS34, encoded by SA11 genome segment 7, is predicted to be a slightly acidic protein (31), and it is the one nonstructural protein that reacts with radiolabeled nucleic acids (RNA or DNA) on Western blots (37). NS34 has consistently been found in complexes isolated from infected cells that contain replicase activity and in complexes formed in vitro in association with NS53 (Au and Estes, unpublished). Again, these data suggest that NS34 may be a component of the viral replicase (145, 247), and, like NS35, further studies are required to define its precise role in the replication process.

NS28. The SA11 genome segment 10 product, NS28, is a nonstructural protein synthesized as a primary translation product of apparent molecular weight 20,000 and then cotranslationally glycosylated (88, 165). The mature glycoprotein is an integral membrane protein of the ER. The amino terminus of this protein contains two sites where N-linked high-mannose oligosaccharides are added (Fig. 8). The locations of these sites of glycosylation and biochemical analyses of the biosynthesis of this protein indicate that NS28 contains a noncleavable signal sequence (35, 88, 165). Studies of the topography of NS28 indicate that the amino terminus of the molecule is maintained in the membrane and the carboxy terminus extends into the cytoplasm of infected cells (23a, 48).

The cytoplasmic domain of NS28 has been proposed to be involved in the morphogenesis of virus particles that mature by budding through the membrane of the ER (89, 252). NS28 has been proposed to act as a receptor to bind subviral particles (possibly single-shelled particles) and mediate their budding into the lumen of the ER (89, 166). Glycosylation of NS28 is required for removal of the transient envelope from budding particles, because enveloped particles accumulate if infected cells are treated with tunicamycin (89, 149, 252). The accumulation of such enveloped particles in cells treated with tunicamycin and infected with the variant containing the nonglycosylated VP7 proves that glycosylation of NS28 is critical for removal of the envelope (252). Kinetic experiments have suggested that single-shelled particles are the precursors to double-shelled particles (166), and a receptor role for NS28 is confirmed by the demonstration that purified single-shelled particles bind specifically to ER membranes containing only NS28 (13, 14, 222a). Further studies of the function of this protein should be helpful to our understanding of the process of virus envelopment in general.

NS26. The identity of the protein product of genome segment 11 as a structural or nonstructural protein has remained unclear until recently. The primary translation product of the SA11 gene 11 is a protein with an apparent molecular weight of 26,000 (88); the predicted molecular weight calculated from the gene sequence is 21,560 for the Wa virus (163, 345) and 21,725 for the SA11 virus (225, 349). This protein was initially given a tentative assignment as a structural protein because it comigrated with a low-molecular-weight protein found in virus particles; however, lack of a high-titer antibody precluded direct proof of this assignment (88, 217). Support for this assignment came from studies with reassortant viruses, which concluded that segment 11 codes for a minor neutralizing antigen (211), and from the finding that the deduced amino acid sequence of this gene displays high sequence variation as expected for outer capsid structural proteins (345). Others concluded this protein was nonstructural (11).

The gene 11 protein is unusual in that it has a very high content of serine and threonine, with these amino acids being present in runs of three or four consecutive residues; these features share similarities with glycophorin (345). Reexamination of the sequence of gene 11 shows that a second out-of-phase open reading frame is present that could code for a protein of 92 amino acids with a predicted molecular weight of 11,012 (225, 349). No evidence exists yet on whether this second protein is produced, but conservation of its start and stop codons and predicted amino acid sequences in all strains sequenced to date are consistent with its being made.

Pulse-chase experiments showed that the gene 11 protein undergoes posttranslational modification following its synthesis (81, 88, 217). Owing to the high content of serine and

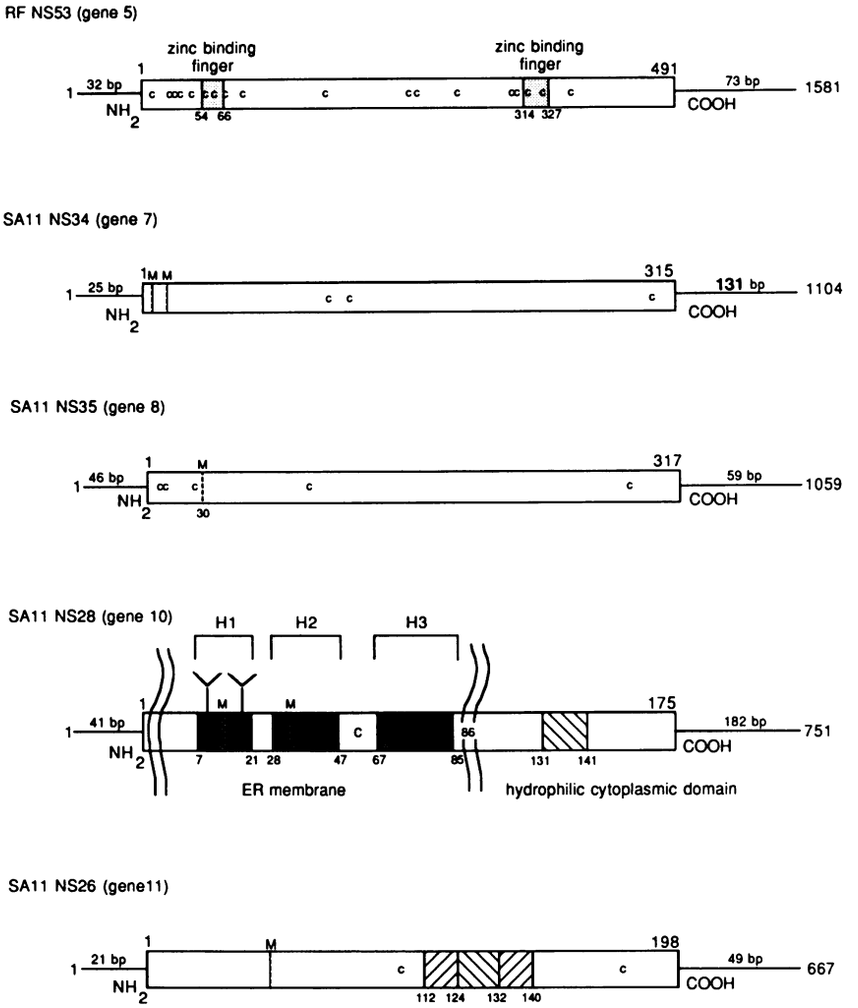


FIG. 8. Features of the nonstructural proteins. These schematics show features as described in the legends to Fig. 3, 4, and 6. In addition, the zinc-binding finger in NS53 (aa 54 to 56 and 314 to 327), known glycosylation sites for NS28 (Y), potential locations of the ER membrane (≀), and regions of extremely high (▧) and relatively high (▨) sequence variation in NS28 and NS26 are shown. Compiled from sequences for the genes which code for these proteins (see Table 3 for references).

threonine in this protein, it has been speculated that it might be modified by O-linked glycosylation. However, attempts to directly confirm this hypothesis have not been successful (349).

Synthesis of the gene 11 protein by using the baculovirus expression system has allowed the production of a high-titer antiserum, which has proved useful in experiments to redress how the protein is modified and whether it is present in virus particles (349). Definite demonstration that this protein is not a third outer capsid protein is important for investigators interested in making a subunit vaccine. Antibody to the baculovirus-expressed protein does not possess neutralizing activity, and it fails to react with virus particles as shown by immunoelectron microscopy, immunoblots, or immunoprecipitation. Instead, these studies indicate that the gene 11 protein is a phosphorylated nonstructural protein and that phosphorylation is not responsible for the mobility shift seen on polyacrylamide gels. The antibody to the baculovirus-expressed protein cross-reacts with viruses of all serotypes, showing discrete foci (viroplasms) in the cytoplasm of infected cells (112a, 349; E. Calomeni and M. K. Estes, unpublished data). This distinct staining could be used as a marker of virus replication.

The gene 11 segment is one of the segments often found to contain rearrangements (215, 250) (see above). Understanding the function of this protein might help us to understand why some segments (usually those encoding nonstructural proteins) undergo rearrangements during the replication cycle (see above). Reevaluation of the deduced amino acid sequences has shown that the high variation among the sequences of different viruses is due to a frameshift in the sequence of the Wa rotavirus at residues 123 to 132. Since this protein is nonstructural, the observed variability between the SA11 and UK sequences in the region suggests that this domain is not essential for functioning of this gene. The presence of a potential mononucleotide-binding sequence (between Gly-31 and Ser-37) in gene 11 and its localization to viroplasms suggest that NS26 may be involved in RNA replication or gene reassortment.

GENE FUNCTIONS IN DIFFERENT STAGES OF VIRUS REPLICATION

The information on the structure of the proteins encoded by the viral genome provides a foundation for understanding the role of each gene in the replication cycle and in virus-

host interactions. Rotaviruses replicate almost exclusively in the differentiated enterocytes in the small intestine, and EM and immunocytochemistry studies of virus replication in intestinal biopsies or tissue sections indicate that the replication process determined from cell culture studies (primarily in monkey kidney cell lines) is similar in intestinal cells. The lack of direct studies of rotavirus replication in intestinal cells reflects difficulties in culturing specific intestinal cells; these difficulties were only recently overcome.

In monkey kidney cells, the replication cycle is fairly rapid, with maximum yields of virus being found after 10 to 12 h at 37°C when cells are infected with virus at high multiplicities (10 to 20 PFU per cell [63, 216, 266]). Similar cycles are found for other virus strains, but the permissiveness of different types of cultured cells to rotavirus infection (and the cytopathogenicity of different virus strains) can vary (15, 57, 98, 132, 169). These different cell lines can be expected to be studied further as efforts to identify the cell receptor and the mechanisms controlling virus-cell interactions expand. Rotavirus infection of cells presumably begins by interaction with a specific receptor, which probably is a sialic acid-containing protein, at least for the simian SA11 rotavirus and the bovine NCDV rotavirus (19, 112a, 177, 357). Human rotavirus strains, having different properties of hemagglutination and cell adsorption compared with some animal strains, may interact with a different receptor (112a). Whether subsequent virus entry (internalization) occurs by receptor-mediated endocytosis or direct penetration of membranes or both remains unclear (64, 169, 201, 253, 265, 321). Understanding the functions of rotavirus proteins in the lytic replicative cycle also will facilitate identification of the cellular or viral factors that result in the establishment of persistent infections. The recent recognition that some (but not all) rotavirus strains cause hepatitis and death when administered to mice with severe combined immunodeficiency raises an immediate interest in identifying the gene(s) critical for this apparent altered viral pathogenesis and cell tropism (279).

Current research efforts to develop new assays to probe the specific steps in the replication cycle based on the expression and interaction of individual proteins and RNAs in in vitro replication systems are expected to rapidly unravel details of the replication cycle. The separate stages of rotavirus replication are not considered here because they have recently been reviewed elsewhere (Estes, in press). However, new information on rotavirus morphogenesis is highlighted because this is such a unique step in the replication of these viruses (Fig. 9). The sites of synthesis or localization of rotavirus proteins have been examined by ultrastructural immunocytochemistry with polyclonal monospecific antibodies or MAbs (254, 277) and by studying the distribution of proteins by immunofluorescence or in subcellular fractions (14, 166, 311, 349). Most of the rotavirus structural proteins and nonstructural proteins are synthesized on the free ribosomes. This conclusion has been drawn on the basis of the lack of predicted signal sequences that would indicate targeting to the ER and the lack of protection of these proteins from digestion in in vitro protease protection studies (89, 165), rather than on the basis of direct analyses of the nascent proteins on free ribosomes. In contrast, direct data show that the glycoproteins VP7 and NS28 are synthesized on ribosomes associated with the membrane of the ER and that they are cotranslationally inserted into the ER membrane owing to the signal sequences at their amino termini (89, 165).

The sites and precise details of RNA replication remain unclear. Cells do not contain enzymes to replicate dsRNA, so the virus must supply the necessary enzymes. Transcripts function both to produce proteins and, once, as a template for production of a minus strand. Once the complementary minus strand is synthesized, it remains associated with the plus strand. The dsRNA segments are formed within nascent subviral particles, and free dsRNA or free minus-strand ssRNAs are not found in infected cells. Methods to separate and analyze the plus and minus strands of SA11 dsRNAs and to synthesize dsRNA in cell-free systems are beginning to identify the proteins associated with replicase activity and the events that control RNA transcription, replication, and protein expression (14, 164, 246–248, 313).

Both transcriptional and translational control of gene expression have been shown to operate in rotavirus-infected cells. This achieves the regulated synthesis of viral proteins during the replication cycle (164). Regulation of transcription has been demonstrated by analyzing the mRNAs made in infected cells treated with cycloheximide. Under these conditions, mRNAs which encode NS53, VP6, NS35, and NS34 were seen. Much higher levels of mRNA which encode VP2 and NS35 also were found to accumulate in cells. Finally, the levels of mRNA present in cells were not directly reflected in the relative amounts of the corresponding protein made, and VP6 and NS28 were produced in large excess over the remaining nine proteins. An excess of minus-strand synthesis was also detected. These results suggest that the replication process is extremely inefficient, at least in cultured monkey kidney (BSC-1) cells.

The localization of several of the viral proteins (VP2, NS35, and NS26) to electron-dense viroplasms, the localization of VP4 and VP6 to the space between the periphery of the viroplasm and the outside of the ER, and the observation that particles emerging from these viroplasms often seem to directly bud into the ER that contains VP7 and NS28 indicate that the viroplasmic inclusions are probably the sites of synthesis of the single-shelled particles (255, 277). Subviral particles form in association with viroplasms, and these precursor particles mature by budding through the membrane of the ER (5, 65, 78, 149). In this process, or after budding, particles acquire their outer capsid proteins.

The morphogenic pathway of rotaviruses is distinctive in that subviral (probably single-shelled) particles, which assemble in cytoplasmic viroplasms, mature by budding through the membrane of the ER and that, during this process, particles possess a temporary envelope (Fig. 9) (for a review, see reference 78). This is one of the most interesting aspects of rotavirus replication, and this unique feature distinguishes these viruses from other members of the *Reoviridae* family. The envelope acquired in this process is lost as particles move toward the interior of the ER, and ultimately, instead of the membrane, a thin layer of protein is found on the outer capsid of mature virions.

A kinetic study of the assembly of VP7 and of other structural proteins into particles was made possible by the ability to distinguish two pools of VP7 in the ER (166). These two pools of VP7 can be separated biochemically by fluorocarbon extraction or by immunological methods (by using two classes of antibodies). One form of VP7, found only on intact particles, is detected only with a neutralizing MAb; a second pool of unassembled VP7 remains associated with the ER membrane and is detectable with a polyclonal antibody made to denatured VP7 (166). The incorporation of proteins into single-shelled particles occurs rapidly, while VP4 and VP7 appear in mature double-shelled particles with a lag time of 10 to 15 min. Monitoring of the kinetics of

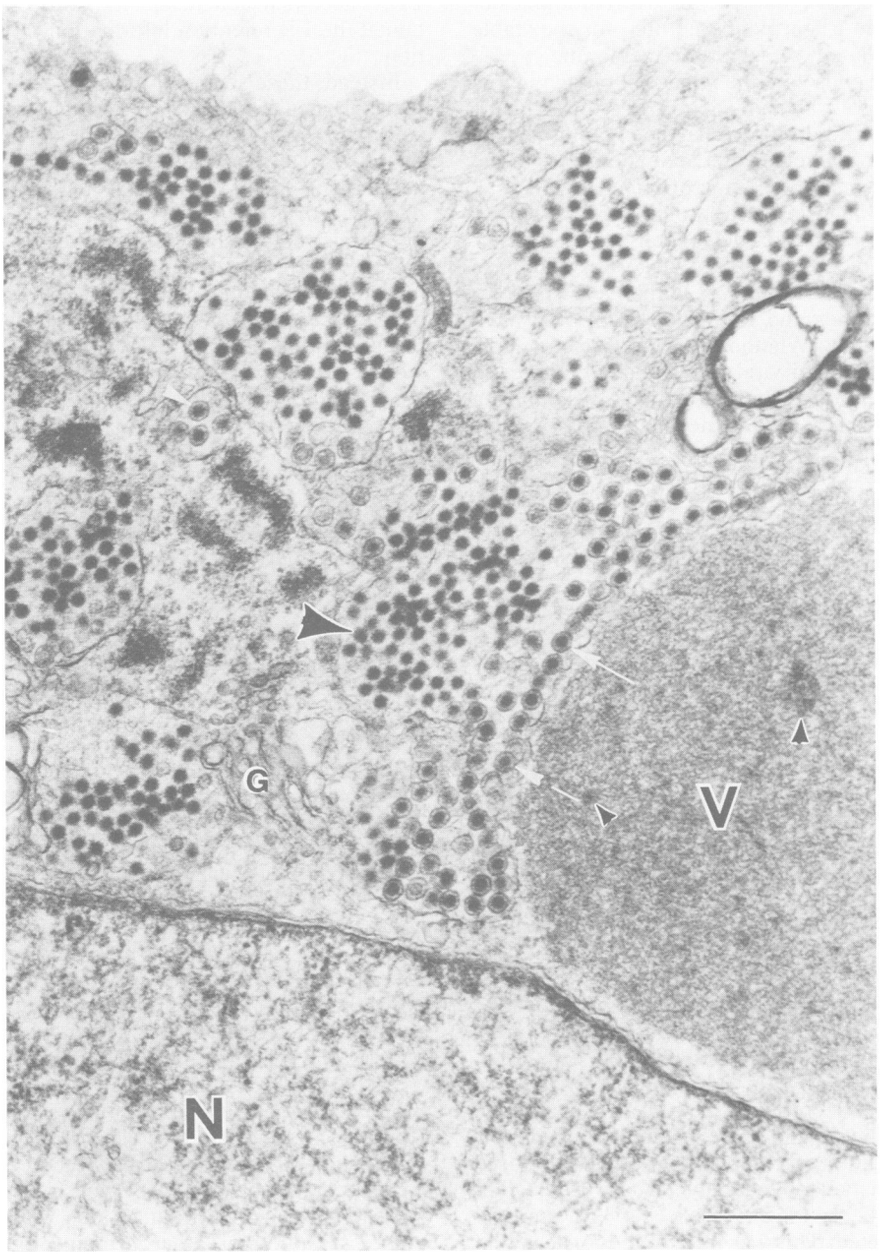


FIG. 9. Unique features of rotavirus morphogenesis. EM of a thin section of SA11-infected MA104 cells. Dense viroplasmic inclusions (V) are abundant in the cytoplasm, often adjacent to the nucleus (N) and in close proximity to regions of the ER. In these structures, inner-core particles (small arrowheads) are assembled. Single-shelled particles located at the periphery of the viroplasm bud into the lumen of the ER, resulting in transient membrane-encapsidated intermediates (white arrows). These membranes are later lost, giving rise to the double-shelled mature virion (large arrowheads). Virus particles are never seen budding in the Golgi apparatus (G) or from the plasma membrane. Bar, 0.5 μm.

processing of the oligosaccharides on each form of VP7 has shown that the virus-associated VP7 oligosaccharides are processed with a 15-min lag compared with that of the membrane-associated form, suggesting that the latter is the precursor to virion VP7. This lag appears to represent the time required for virus budding and outer capsid assembly (166).

Rotavirus maturation is apparently a calcium-dependent process, since it was observed that virus yields are decreased when produced in cells maintained in calcium-depleted medium (300). In the absence of calcium, viruses produced are reportedly exclusively single shelled and budding of virus particles is not observed (299). Reduced levels of only VP7 were observed owing to the preferential degradation (not to the impaired synthesis) of VP7 (299). An interesting result in these studies was that unglycosylated VP7 made in the presence of tunicamycin (but not mature VP7) was relatively stable in a calcium-free environment. Unfortunately, the role of calcium in morphogenesis was not completely revealed by these studies. It is possible that calcium stabilizes or modulates folding or compartmentalization of the newly glycosylated VP7 for subsequent assem-

bly into particles or perhaps that calcium deprivation destabilizes the ER or other ER proteins required for the stable association of glycosylated VP7 with the membrane.

The infectious cycle ends with the release of progeny virus by cell lysis, as observed by thin-section EM (5, 55, 221). Extensive cytolysis during infection has been confirmed by biochemical analyses of infected cells, and alterations in the permeability of the plasma membrane of infected cells may result in release of cellular and viral proteins (227). Despite cell lysis, all single-shelled particles and many double-shelled particles remain cell associated, suggesting that these particles interact with some structures within cells. Interactions with cell membranes and the cell cytoskeleton have been demonstrated (227). Whether the cytoskeleton provides a means of transport of viral proteins and particles to discrete sites in the cell for assembly or acts as a stabilizing element at the assembly site for newly budded virions remains to be determined.

STUDY OF ROTAVIRUS PROTEINS TO PROBE EUCARYOTIC PROTEIN TARGETING AND OLIGOSACCHARIDE PROCESSING

The exclusive rough endoplasmic reticulum (RER) maturation site of the rotaviruses has been exploited to probe the behavior of membrane glycoproteins which have the RER as their final destination in the cell. The ER contains a mixture of proteins with multiple destinations. Some remain in the ER as permanent residents, whereas others are exported to the Golgi apparatus for subsequent distribution to the cell surface, lysosomes, or (in many cell types) secretory storage vesicles. The site of maturation of rotaviruses in the ER and the fact that no complex carbohydrate is detectable on viral VP7 show that these proteins are not transported to the Golgi apparatus. Studies of the processing of these glycoproteins have been useful in finding how protein targeting is controlled and in determining whether specific signals direct protein retention in the ER.

The first evidence suggesting that specific signals mediate protein retention in the ER came from studies of the expression of a series of deletion mutants of VP7 in COS cells (259). Deletions in aa 47 to 61 in VP7 (in the second H2 hydrophobic domain) resulted in secretion of a truncated membrane protein into the medium, and this protein obtained new modifications (the addition of complex carbohydrate, based on its becoming resistant to endoglycosidase H). Other variants lacking aa 42 to 61 or 43 to 61 were similarly glycosylated and secreted. This was interpreted to mean that the second hydrophobic domain contains a positive signal for ER location and a membrane anchor function. However, an alternative interpretation would be that the truncated protein is secreted simply because it is in the aqueous phase and is not membrane bound.

Subsequent studies with additional deletion mutants that contained either the first or the second hydrophobic region showed that either of the hydrophobic regions can direct the transport of VP7 across the ER membrane, but the nature of the anchor and targeting signal in VP7 was not elucidated (350). Identification of the site of cleavage in VP7 as occurring after residue 50 indicated that both amino-terminal hydrophobic domains are removed from the mature protein (257, 316). Since VP7 normally remains anchored after processing, these results implied that residues distal to residue 50 (i.e., the region from aa 51 to 61 that was present in wild-type VP7 but missing from the secreted mutant lacking aa 47 to 61) must be involved in retaining VP7 in the ER. The initial conclusion that the H2 domain itself contained the ER retention information thus required modification.

Instead, these seemingly conflicting results are consistent with the idea that aa 47 to 61 might be necessary, but not sufficient, for ER retention. This idea has been tested directly by studying the expression and secretion of chimeric genes (257, 315). Poruchynsky and Atkinson constructed chimeric genes by fusing the first 63 codons of wild-type VP7 or of constructs with deletions of residues 1 to 14 or residues 51 to 61 to the mouse salivary α-amylase gene, a secretory protein. The fusion junction in these constructs was located at the exact mature terminus of amylase, and the chimeric proteins from all three of these constructs were secreted, based on their being present in the media. One weakness of these studies was that there was no way to determine whether the presence of the protein in the media was due to active transport or to cell lysis, because the chimeras were not glycosylated (257).

A study of a second series of VP7-amylase chimeras in which the VP7 contribution extended to aa 111 resulted in retention of amylase within the ER, and this protein retained enzymatic activity. These results, therefore, suggested that two regions of VP7 mediate its retention in the ER. The first lies within the region spanning aa 51 to 61, and the second lies in the region spanning aa 62 to 111, the latter containing the single glycosylation site for VP7. Both regions are apparently necessary for retention, although neither by itself is sufficient for retention (257).

Different conclusions on the residues of VP7 needed for ER retention were reached in an independent study in which amino acid residues 30 to 64 of VP7 were exchanged with the signal peptide from the influenza virus hemagglutinin (315). These constructs, which maintained the usual cleavage site for VP7, caused secretion of VP7. Because these HAF VP7 constructs contained both regions (aa 51 to 61 and 62 to 111) proposed (as described above and in reference 257) to be necessary for VP7 retention, these regions alone are clearly not sufficient for VP7 retention. Studies with other chimeric proteins indicate that interactions between the cleaved signal peptide (the H2 domain) and other downstream sequences in VP7 are required, directly or indirectly, for retention of this protein in the ER membrane (315). This is the first demonstration of a function for a cleaved signal peptide.

The method by which VP7 is retained in the ER remains an interesting question. VP7 does not contain the discrete peptide Lys-Asp-Glu-Leu, which was recently found to confer retention of other proteins to the ER (226). The topography of VP7 in the membrane also is not well understood. Based on its resistance to digestion with proteolytic enzymes, VP7 is not a membrane-spanning protein (89, 165). However, VP7 is membrane associated since it remains membrane bound after high-salt treatment and release of its microsomal contents at high pH by sodium carbonate treatment (165). It is possible that VP7 is retained in the ER by conformational properties that mediate membrane attachment or by oligomerization with itself or other specific cellular proteins.

These analyses of processing of VP7 with gene 9 mutants produced by site-directed mutagenesis show the power of this approach to the correlation of gene structure and function. These studies of COS cells transfected with mutants containing either both initiation codons or only the second initiation codon all clearly indicate that the first AUG is probably not translated and that the mature VP7 lacks both hydrophobic domains and contains an amino-terminal Gln-

51 (257, 316). These results are of interest for investigators using strategies to produce authentic VP7 in expression vectors for testing as potential vaccines (see below), and they also reemphasize the puzzling question of why both initiation codons that each precede a hydrophobic domain are retained in all VP7 genes sequenced so far. Comparisons of the amino acid sequences of VP7 are striking when the conserved and variable amino acids found upstream of the cleavage site prior to Gln-51 are examined. The first 8 aa of VP7, both initiation codons, leucine 23 and lysine 25 at the end of the H1 domain, and glutamine 51 following the H2 domain (and shown to be the major site of cleavage of the mature protein) are strictly conserved. These conserved regions are consistent with the idea that both initiation codons are used. In contrast, extensive variation is seen in the H2 domain (Fig. 6). One wonders what factors cause such variation in this domain. Perhaps these changes compensate for changes downstream in other amino acids (between aa 62 and 111) with which H2 may interact (257, 315).

Analysis of the synthesis and expression of a series of altered gene 10 cDNAs has been used to localize the sequences involved in translocation of the nonstructural glycoprotein NS28. A series of mutations in the coding regions for the three hydrophobic domains (H1, H2, and H3) of this protein were constructed and analyzed by in vitro translation and by transfection in vivo. In vitro, all three hydrophobic domains associated with microsomes. However, glycosylation and proteolysis of wild-type and mutant forms of NS28 indicate that the wild-type protein is anchored in the membrane only by the second hydrophobic domain, leaving approximately 131 residues exposed on the cytoplasmic side of the membrane (23a). These studies extended initial studies on the topology of NS28 in microsomal membranes (48) by suggesting that the cytoplasmic domain is larger than originally thought and by proposing a model in which the second (H2) hydrophobic domain is the membrane-spanning domain rather than the third (H3) as proposed earlier. Although both papers confirm that the H3 domain is protected from digestion with various proteases, analyses of the mutant molecules indicate that protease sites upstream of the H3 domain are available for cleavage. It is therefore concluded that H3 is merely resistant to cleavage by being embedded or protected by interactions with the cytoplasmic side of the membrane (23a). Site-directed modification of the cytoplasmic domain of the protein will no doubt help to better define the domains of NS28 involved in receptor-ligand interactions.

Studies of the processing of the oligosaccharides on the rotavirus glycoproteins have also been useful in probing the types of α-mannosidases specific to the RER. Initial pulse-chase experiments showed that the oligosaccharides on the rotavirus glycoproteins are trimmed (89, 165); $Man_8GlcNAc_2$ and $Man_6GlcNAc_2$ were found to be the predominant intracellular species detected after a 5-min pulse with [^{3}H]mannose and a 90-min chase, and VP7 on mature virus was processed to $Man_5GlcNAc_2$ (165). Although removal of the outer glucoses and at least one mannose residue had been shown to occur on nascent chains of vesicular stomatitis virus G protein in the RER (12), the location, linkage specificity, and total number of the different α2-mannosidases acting during the remaining processing reactions were not previously clear. Evidence had been presented for both RER and Golgi apparatus activities (156). In contrast, the subsequent steps of carbohydrate modification, including the addition of *N*-acetylglucosamine residue, removal of α1,3- and α1,6-mannoses, and transfer of terminal sugars, were known to be localized to the Golgi complex (104). Studies of the processing of the rotavirus glycoproteins provide evidence that α1,2-mannosidase activities responsible for the formation of $Man_8GlcNAc_2$ and $Man_6GlcNAc_2$ on VP7 and $Man_8GlcNAc_2$ on NS28 reside in the ER (165, 258). Trimming of glycosylated VP7 (but not of vesicular stomatitis virus G protein) was also able to continue in the presence of the energy inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). These results suggest that the Man_8-forming enzyme is in a functionally distinct compartment to the Man_8- and Man_6-forming enzymes. In addition, it suggests that an energy-sensitive translocation step within the ER may mark the divergence of the processing pathways of glycoproteins which have distinct final destinations in the cell (258).

GENETIC APPROACHES TO UNDERSTANDING ROTAVIRUS GENE FUNCTION

Studies with rotavirus mutants and reassortants have only begun to contribute to our understanding of rotavirus gene structure and function. Temperature-sensitive mutant collections have been isolated from several rotavirus strains, including SA11, UK, and RRV (105, 138, 266, 268). The most extensive mutant collection is for SA11, for which 10 of the expected 11 reassortment groups have been identified, and 6 of these have been assigned to genome segments (124). The phenotypes of some of these mutants are consistent with functions inferred from biochemical data or recent sequence information (reviewed above). For example, mutant groups B and C, which mark genome segments 3 and 1, respectively, are both defective in the synthesis of ssRNA and dsRNA (124). Since these proteins are suspected to be part of the transcriptase, biochemical and sequence analyses of these mutants may now permit dissection and localization of specific enzymatic domains on VP1 and VP3. The availability of full-length cloned DNAs for the rotavirus genes has stimulated the desire to learn how to selectively insert genes into rotavirus-infected cells and rescue this information in an infectious particle. Success in these experiments will have a major impact on rotavirus research. One approach to this idea is to coinfect cells with an mRNA (or a cDNA that can express an mRNA) and a temperature-sensitive mutant and to then select particles from the progeny that would contain the wild-type gene. Information on the genetic interactions between temperature-sensitive mutants (or two wild-type viruses) is available that is relevant when trying to produce reassortants or to determine whether a specific gene can be rescued.

The genetic interactions between pairs of temperature-sensitive mutants occur in an "all-or-none" fashion, as expected for recombination by a mechanism of reassortment of genome segments (266). Analysis of the factors that affect genetic interactions has shown that recombinational frequency is affected by the multiplicity of infection and the time of infection (269). The maximal or near-maximal recombination frequency is obtained at the earliest times when significant recombination can be detected; this suggests that recombination occurs early in the infectious cycle and that there is a single round of mating. Recombination is efficient at nonpermissive temperatures, and mutants from all recombination groups can interfere with the growth of wild-type virus at both permissive and nonpermissive temperatures. An independent study of the factors that may affect reassortant formation found that no selection of genomic RNA segments was detectable before or during virus particle assembly in coinfected cells. In addition, reproducible selec-

tion of reassortants was observed with specific pairs of parental viruses. Therefore, selection of specific reassortants following coinfection is apparently due to differences in the infectivities of progeny viruses and not in their assembly. This implies that reassortant infectivity is a function of the parental origin of specific genomic segments and that selection of surviving reassortants appears to occur because certain reassortants grow better than other reassortants (123, 348).

Reassortment of genome segments among the progeny of in vitro mixed infections has been exploited to derive reassortant viruses with a desired subset of genome segments from each parent. Such reassortants have been used to correlate functions with specific genome segments. In addition to allowing the assignment of temperature-sensitive mutants to a specific genome segment, this powerful approach has identified a number of biologic properties that cosegregate with specific genome segments (reviewed above and below). This genetic approach to probing gene function will continue to be used to map phenotypic differences that are identified between two parental viruses in the future.

Characterization of the progeny of cells coinfected with two strains of rotaviruses has shown that phenotypically mixed viruses (pseudotypes and mosaics of the capsid protein VP7) readily form. This was demonstrated by showing that the progeny were able to be neutralized by MAbs specific for the VP7 of each of the coinfecting strains (347). This result has practical implications, because it suggests that selection of reassortants (containing specific VP4 and VP7 proteins) by using neutralizing antibodies may be of little value unless these antibodies are added only to the overlay agar during plaque formation.

Recent studies in which reassortants were used to map biologic properties of rotavirus genome segments have shown that the criteria needed for firm conclusions from studies with reassortants are more rigorous than formerly realized. For example, early studies which examined the gene segments responsible for inducing neutralizing antibody identified only one genome segment (8 or 9, depending on the virus strain) with this property, but in those studies no reassortants containing a gene segment 4 from a human virus was able to be cultivated and characterized (138). Subsequent studies revealed that genome segment 4 also possesses this property, and this highlights the importance of analyzing reciprocal reassortants when using the genetic approach for phenotypic mapping.

In addition, unknown common properties of parental viruses may influence the interpretation of genetic studies of reassortant viruses. For example, two bovine viruses (UK and NCDV) were eventually found to possess related VP7 antigens, but their VP4 antigens are distinct (139a, 153). Therefore, properties related only to the distinct genome segment 4 of each of these two parental viruses would be able to be dissected by reassortant analyses. Since the common properties of parental viruses may not be known in advance, it is useful to confirm results by using different parental strains. The identity of naturally occurring intertypic rotavirus strains (152, 153, 214, 224) and the direct demonstration that rotavirus genes reassort at high frequency in vivo (123) and in vitro (131, 138, 266) highlight the fact that the genetic background of any rotavirus isolate cannot be assumed. This point has been further illustrated in recent analyses of reassortants which indicate that the phenotype of specific reassortants is altered depending on the parental strain used for analysis (56).

ROTAVIRUS GENES INVOLVED IN PATHOGENESIS AND IMMUNITY

Details of the current information on the genes involved in pathogenesis and immunity have been reviewed recently (209), so we shall highlight only the areas where confusion exists. Most of the direct information on the genes involved in pathogenesis and immunity has been obtained by using a wide variety of animal models and a number of different rotavirus strains. Studies with these animal models must be distinguished in terms of whether they evaluate the role of active (rabbits, gnotobiotic piglets to 3 months of age, calves to 3 weeks of age) or passive (mice, lambs, and calves) immunity and limited (abortive) or extensive viral replication. Results with all these models and conclusions from studies of natural infections in children indicate that protective immunity does develop after rotavirus disease; less information is available on the duration of this immunity, whether protection from reinfection is possible, and how to best induce protective immunity.

Virus virulence has been attributed primarily to properties of genome segment 4. Offit et al. (238) made reassortants between two heterologous rotaviruses (SA11 and NCDV) and mapped differences in the 50% diarrhea dose in the neonatal mouse model of gastroenteritis exclusively to gene segment 4. Understanding the nature of heterologous host-virus infections was of interest since viruses of animal (heterologous) origin are currently being explored as vaccines for use in humans. In the mouse model, replication of these heterologous viruses is limited and usually abortive (270), and disease can be evaluated only in neonatal animals (<14 days of age). Because of this and because the difference in diarrheal dose of the parental strains used to map the diarrheal gene was only 50-fold (238), further studies with more virulent homologous viruses in this and other animal models are warranted. Such studies are needed to confirm whether additional genes influence disease induction and other properties of virulence, such as replication or virus transmission. The recent ability to cultivate and quantitate murine rotavirus strains will facilitate such studies with neonatal mice (140), and studies with other older animals (rabbits, calves, and gnotobiotic piglets) are also possible with several cultivatable virus strains (69, 151, 334, 355).

Genome segment 4 has also been implicated to be important in virulence on the basis of comparisons of the amino acid sequences of viruses originally isolated from newborn babies with asymptomatic infections and older babies with symptomatic infections (126, 127). Further studies are required to determine the validity of this hypothesis and to determine the relative roles of host and viral factors in determining whether infections of newborns (and older children) result in symptomatic or asymptomatic illness. New evidence shows that symptomatic infections do occur in newborns, that asymptomatic infections may occur much more frequently than previously realized, and that the appearance of new virus types may cause unexpectedly severe illness in populations generally thought to be nonsusceptible (17, 77, 112; D. O. Matson, M. K. Estes, J. W. Burns, H. B. Greenberg, K. Taniguchi, and S. Urasawa, submitted for publication). These new findings emphasize the current limitations of our understanding of the virulence properties of the rotaviruses and their epidemiology.

Analysis of the sequences of genome segment 4 of two virulent calf viruses and of the avirulent RIT 4237 vaccine strain has recently been completed (235). Comparisons of these sequences show no changes in the trypsin cleavage

area, and only one amino acid difference (a change of glutamine 23 to lysine) between the virulent Cody strain of NCDV and the avirulent RIT vaccine strain was found. These results indicate that either aa 23 (outside the cleavage region) plays an important role in virulence or, more likely, a gene (or genes) other than genome segment 4 contains mutations responsible for attenuation of the RIT strain of NCDV. Further studies on the gene(s) responsible for virulence are clearly warranted.

As mentioned above, protective immunity to rotavirus gastroenteritis can be induced. However, what constitutes the protective immune response and exactly how to induce it have not been completely defined. Difficulties in interpreting the existing data on immunity include the following: (i) an inability in studies with children to know the previous exposure to rotavirus and problems with dissecting the effects from maternally acquired antibody from those of active primary or secondary infections; (ii) a lack of studies of active immunity in animal models which mimic infections in children on the basis of virologic and immunologic parameters; (iii) seeming conflicting results on which in vitro parameter is the best to use to measure or determine homotypic or heterotypic protective immunity in vivo; (iv) lack of knowledge or systematic consideration of how different virus strains, different virus dosages, and different host factors may influence results; and (v) difficulty in obtaining antibody-negative animals and failure to perform studies in proven antibody-negative animals. Fortunately, the recent expansion of our knowledge of the antigenic and molecular properties of the rotaviruses and new expanding sources of molecular probes, reassortants, and immunologic reagents will help dissect the immune response in future studies.

The proteins involved in inducing a protective immune response have been studied with reassortants containing different types of VP4 and VP7. Mouse dams orally hyperimmunized with a reassortant rotavirus containing the VP4 of one serotype and the VP7 of another serotype confer to their progeny passive protection against challenge with either parental rotavirus (239). Similar results were found following primary infection of 4- to 5-day-old gnotobiotic piglets with reassortant viruses containing distinct types of VP4 and VP7 and challenge with two serotypically distinct virulent viruses 3 weeks later (151). These results indicate that antibodies to VP4 and to VP7 are each associated with resistance to diarrhea. These results are of general importance, because they suggest that a reassortant (or selected virus strains with a particular gene constellation) may be able to induce broader protection than either of the two parental virus strains. In addition, these reassortants were able to induce heterotypic immunity, although this was not seen after immunization with either of the parental virus strains. The general conclusions of these studies have been confirmed by showing that passive administration of neutralizing MAbs to VP4 and VP7 also can provide protection against challenge in the mouse model (209, 243).

Several studies have raised doubts about the importance in protection of the differences in neutralization antigens measured by in vitro plaque reduction assays, and mechanisms other than neutralizing antibody have been proposed to be important in protection. Initial studies failed to show heterotypic protection in gnotobiotic calves (354). However, more recently, protection between different serotypes has been seen, but this was dependent on the virus strain used for immunization. Unexpectedly, the plaque reduction neutralization titers of antibody in serum and coproantibody did not predict a state of protection against the challenge virus (355). The question of whether an infection with an avirulent rotavirus strain can protect against subsequent rotavirus disease when the avirulent virus is related (or only poorly related) to the challenge virus by neutralization has also been asked. Results with calves indicate that one avirulent virus can provide protection from disease without inducing high levels of neutralizing antibody in serum (40). Such studies suggest that our knowledge of the immune response to rotavirus infections is clearly incomplete. Efforts to evaluate the role of cell-mediated immune responses and nonimmune factors in the resistance to infection and disease may help clarify this current confusion (182, 242, 278, 336).

Other approaches, examining the immune responses to specific proteins, are directed toward finding the proteins important in protection. A method to measure immune responses to specific epitopes on rotavirus proteins has been developed that measures the ability of a serum to block the binding of a specific MAb to virus (22a, 301). This and similar competitive assays show great promise in being able to determine what responses are standard and whether the response to a particular epitope can be associated with protection following an infection in an individual. However, more data are needed on the sensitivity and specificity of this assay. For example, it will be important to know that antibody responses to distinct epitopes situated closely on VP4 or VP7 do not compete with the specific binding of the MAb being used in the assay. Alternative assays to measure antibody responses to specific proteins are using proteins produced in expression vectors (see below).

Electrophoretic analysis of viral proteins recognized in immunoprecipitation reactions is a third method to determine the immune response to specific proteins (69, 324). This method has the advantage of allowing responses to proteins other than structural proteins to be easily recognized, and correlation of primary responses with the time when animals become protected from challenge may help dissect which responses are protective. Studies analyzing the serum and mucosal antibody responses are only beginning to be performed (141, 199, 291). Interestingly, both children (responding to natural infections) and rabbits (responding to a known primary infection) develop antibodies to a number of proteins, but serum samples with neutralizing antibody do not necessarily possess detectable antibody to VP7 (324; M. E. Conner, M. Gilger, D. Y. Graham, and M. K. Estes, manuscript in preparation). Strong reactivities are detected to several of the nonstructural polypeptides, indicating that these are good markers to document virus replication in the gastrointestinal tract; these responses to nonstructural proteins raise the question of whether they also play a role in protection. Finally, differences in responses of hyperimmune and postinfection serum have been noted (302), and these demonstrate that studies comparing the immune response to parenteral immunization and intestinal infection will be worthwhile. Such studies may help us learn how to induce responses to different proteins or domains within proteins, as is being learned for responses to poliovirus vaccination (358).

Additional analyses of parental and reassortant viruses will continue to be useful to dissect the genes involved in virulence, to determine the mechanism by which VP4 (and possibly other gene products) affects virulence, and to determine the relative importance of specific and nonspecific immune and nonimmune factors in mediating protection from infection and disease. Such information will have practical potential until an effective rotavirus vaccine is

developed and will be of basic interest in expanding our understanding of the mechanisms controlling viral replication in the gastrointestinal tract.

IMPACT OF MOLECULAR BIOLOGY ON VACCINE DEVELOPMENT

Active Immunization

Epidemiologic studies showing the significant mortality and morbidity caused by rotavirus disease in individuals in developing and developed countries, respectively, focus the need for an effective vaccine. Since life-threatening and clinically significant disease occurs in children less than 2 years of age, current vaccines are aimed at preventing clinically significant diarrhea (and not infections) in this target population. The first vaccines to be tested in children have been live vaccines, because they are expected to induce more effective local mucosal immunity and greater duration of immunity than killed vaccines (52, 86, 175, 343).

The initial live candidate vaccines to be developed have been from animal strains. Three candidate vaccines (RIT 4237, a bovine serotype 6 virus; RRV MMU 18006, a rhesus monkey serotype 3 virus; and WC3, a bovine serotype 6 virus) have progressed to field trials. These vaccines are not representative of the most common serotypes found in humans, but they were developed with the assumption that heterotypic immunity would provide protection from disease with human viruses (175). The bovine strain (RIT 4237) showed good efficacy when tested initially in developed countries, but failed to provide protection when tested in developing countries; it has now been removed from further testing (351). A subsequent field trial of RIT 4237 in Lima, Peru, showed that this vaccine afforded 58 to 75% protection against the more severe rotavirus illness if given in more than one dose, and better protection was found against disease caused by serotype 1 viruses than by serotype 2 viruses (191). This vaccine trial provided important insights by defining the requirements for performing a successful vaccine trial in a developing country (191). It also highlighted the need to provide protection against multiple rotavirus serotypes and the possible need for multiple doses to obtain adequate protection.

Surprisingly, most vaccine trials have been performed in areas where the natural challenge viruses were of one or two serotypes. Increasing information on the geographical distribution and changes in the prevalence of rotaviruses indicates that it will not be possible to predict which serotype(s) will be more common at a given time in a given geographical location (342a; Matson et al., submitted). Because of this, none of the current vaccine candidates have really been given a thorough evaluation, and possibly a live polyvalent vaccine will be necessary to account for expected changes in serotype distribution.

The RRV MMU 18006 vaccine has undergone fairly extensive testing. It has been shown to be immunogenic and slightly reactogenic, and it has shown efficacy in field trials to natural infections with serotype 3 challenge but not serotype 1 challenge (52). These results, together with the previous studies of the RIT 4237 vaccine, suggest that serotype-specific immunity may be required for satisfactory protection against rotavirus diarrhea. This conclusion agrees with epidemiologic data, which showed that natural protection against rotavirus gastroenteritis is related to concentrations of serum antibody against homotypic virus and that the levels of heterotypic antibodies are generally below the threshold level needed for protection (58). Studies of the active immune response in known seronegative animals also indicate that the primary neutralizing immune response is homotypic (93, 310). This suggests that demonstration of heterotypic responses in vaccinated children may reflect measuring responses in children who had been previously primed by unrecognized infections (93, 309) or who are infected by a virus strain whose outer capsid proteins contain epitopes shared by several other virus strains (73).

Preliminary efficacy testing of the bovine WC3 vaccine showed that this virus strain provided protection against disease with heterologous human serotype 1 virus infections when tested in the United States (60). These results are promising (as were the initial RIT 4237 trials), and only additional field trials will determine whether this vaccine will prove efficacious in other settings, including developing countries. If it does, further characterization of this bovine virus strain will be of interest to determine the mechanism by which it induces heterotypic protection.

Other approaches to developing candidate vaccines are under way. Reassortants containing a single gene which codes for the outer capsid glycoprotein from human virus serotypes on a rhesus rotavirus background have been produced as possible candidate vaccines to induce homotypic immunity to the four human serotypes (223). These strains are now in phase I trials to evaluate their reactogenicity and immunogenicity and to determine whether administration of a polyvalent vaccine will affect "takes" of individual strains (109, 143).

The possibility that viruses that have become endemic in neonatal nurseries are attenuated, because they cause asymptomatic infections, and that they might be useful as human virus vaccine strains is also being considered. This idea is based on an epidemiologic study showing that children infected with these strains as neonates had no severe disease with subsequent rotavirus infections during their first 2 years of life (28). Therefore, neonatal vaccination with such strains may induce protective clinical immunity. However, there is currently no direct evidence from studies in animal models or volunteers that these viruses are actually attenuated. This idea needs further confirmation, since other recent studies suggest that reinfections may give rise to an equally or even more severe illness (193).

The availability of cDNA copies of many of the rotavirus genes has opened the possibility of using live expression vectors or subunit vaccines of individual proteins synthesized from these clones as recombinant vaccines. Possible approaches have been discussed elsewhere (33, 95). The rotavirus outer capsid proteins (VP4, VP7) have been expressed alone or as fusion products using bacterial expression systems (8, 9, 110, 219). Expression of about 45% of the SA11 VP4 in a thermoinducible expression system resulted in the synthesis of hybrid MS2 polymerase-VP4 protein which was antigenic on the basis of immunoblot reactivity with hyperimmune guinea pig anti-SA11 serum. Immunization of mice with this fusion protein induced antibodies which neutralized virus with a mean titer of 1:1,200. Although these titers were at least 20 times lower than those obtained following immunization with complete virus particles, the hemagglutination inhibition titers in each type of serum were similar. Heterotypic neutralization titers were not reported. The synthesized protein was aggregated, and it is not known whether this had an effect on the immunogenicity of this protein. VP4 expressed from baculovirus recombinants also is antigenic and immunogenic (202; Estes, unpublished).

Several different constructs of VP7 have been expressed in bacteria (8, 110, 219). VP7 expressed alone was toxic (219), whereas VP7 expressed as a fusion protein with β-galactosidase was not. Neutralizing antibody of relatively low titer (1:100 to 1:1,600 for SA11 VP7 [8]; 1:300 for UK bovine VP7 [219]) has been induced, but antibody to expressed NCDV VP7 did not possess neutralizing activity (110). Examination of the immunogenicity of VP7 produced by using a vaccinia virus expression system indicated that neutralizing antibodies with low titers (1:40 to 1:640) were induced and that these were serotype specific when tested in plaque reduction assays or by ELISA (6). In immunoblots these sera reacted with denatured VP7 from serotypes 1, 3, and 4, and the lack of reactivity with serotype 2 was suggested to be due to blocking of a possible cross-reactive site at aa 149 by carbohydrate in these viruses.

A high-yielding baculovirus expression system has also been used to successfully produce 9 of the 11 rotavirus gene products (94, 202; Cohen and Estes, unpublished). The proteins synthesized in this system possess native conformation on the basis of reactivities with available MAbs and polyclonal antibodies and biochemical comparisons with their authentic counterparts made in mammalian cells. VP6 expressed alone forms trimers and capsomerlike structures (94). Neutralizing antibody is induced by immunization of guinea pigs with VP7 (Estes, unpublished). Future efforts will determine whether an effective subunit vaccine can be produced by immunization with these antigens singly and in combination. Since none of the expressed proteins induce neutralizing antibodies with equivalent titers to those produced by immunization with viral particles, it is possible that combinations of proteins will be required to achieve proper conformation and the best vaccination results.

Successful expression of the rotavirus proteins is only the first step toward making an effective subunit vaccine to protect against rotavirus gastroenteritis. The following challenging questions must now be considered. (i) Will it be possible to induce a protective mucosal immunity by oral vaccination with a nonreplicating antigen? (ii) Can novel delivery systems or adjuvants be established to ensure proper antigen presentation in the gastrointestinal tract? (iii) Can long-lasting immunity be induced? (iv) Can subunit vaccines safely and effectively immunize malnourished and/or immunocompromised children who normally may develop persistent infections?

The potential for improving the immunogenicity of expressed rotavirus peptides has already been demonstrated by including peptide determinants known to induce T helper cells in mice (30). Knowledge of the specific rotavirus proteins that induce intestinal cytotoxic T cells and stimulate the uptake of viral antigen by Peyer's patches and knowledge of the role of cell-mediated immunity in protection against, recovery from, or pathogenesis of rotavirus disease may permit the stimulation of more rapid recovery from infection. Studies addressing these questions have been initiated (76, 242, 278). Future studies addressing these questions and the testing of live expression vectors to deliver antigens directly to the gastrointestinal tract are expected to be fruitful.

Passive Immunization

Alternative approaches to active vaccination to prevent rotavirus gastroenteritis are also being considered. In animals in which the young receive immunoglobulins from colostrum and milk instead of by transplacental transfer, vaccination of dams can raise colostral antibody levels to sufficiently high titers that the young animals are passively protected through the time of maximum sensitivity to infection (289). Success has been achieved with this approach in calves (22a, 290, 308). It has clearly been shown that in these animals, vaccination with live or inactivated vaccines boosts the immune response in naturally primed animals, and the resulting heterotypic lactogenic immune response has been shown to be dependent on the preexposure history of the animals (43, 309). Because of the expected lower cost of production and safety testing of subunit vaccines and the ability to use inactivated and heterologous viruses to boost lactogenic immunity, testing of the available expressed proteins in such systems may be worthwhile.

The possibility of ameliorating rotavirus infections in children by the administration of cow's milk containing such heterotypic antibodies is also being evaluated. Although such treatments will not be recommended in developing countries, where breast-feeding needs to be encouraged, they have been used to stop prolonged rotavirus excretion in immunocompromised and normal children (146, 198). Although it remains unclear whether such therapy will significantly decrease the incidence of diarrhea in immunocompetent children, it is possible that such treatment will be useful in certain settings (e.g., day-care centers) where diarrhea is a continuing problem and decreased shedding might help prevent transmission.

CONCLUDING REMARKS

The new information learned about the structure and function of rotavirus genes and their proteins in the last 5 years is impressive in its amount and its significance in helping to understand the epidemiology, pathogenesis, replication, and molecular biology of these viruses. Future studies to understand how trypsin cleavage mediates the selective internalization of the viral capsid into cells and how the nonstructural proteins function in the replication cycle should elucidate the mechanisms by which the virus replicates and encapsidates one of each of its 11 genome segments into nascent particles. This information, in turn, may allow the manipulation and encapsidation of any RNA segment. Further studies of rotavirus morphogenesis should help clarify the virus-budding process in general. With the fundamental information on the structure of the genes and their proteins reviewed here, research efforts can be directed toward unraveling the biology of these viruses and their interactions with the hosts they infect. Studies to identify the cellular receptor, to dissect the immune or nonimmune responses to specific proteins that are important for a protective immune response, and to learn how to induce these responses can be expected. Finally, further studies of the properties of viruses that circulate over time or that reinfect children should shed light on how these viruses evolve and help determine whether this evolution will complicate needed vaccination programs.

ACKNOWLEDGMENTS

Work in our laboratories is supported in part by Public Health Service grants DK30144, AI20649, and AI24649 and training grant CA09197 from the National Institutes of Health (M.K.E.) and by European Economic Community grant BAP 156 F (J.C.).

Computer analyses were performed with the programs and kind staff assistance of the Molecular Biology Information Resource, Department of Cell Biology, Baylor College of Medicine, and Molecular Biology Programs, Institut National de la Recherche

Agronomique. We thank our many colleagues who contributed manuscripts prior to publication and who generously read and critiqued this review. We also thank our students, postdoctoral fellows, and technicians whose creativity and experimental expertise continue to stimulate our work. Finally, we thank Katie Pierson and Caroyl Robinson for patient and skillful preparation of this manuscript.

LITERATURE CITED

1. **Aboudy, Y., I. Shif, I. Zilberstein, and T. Gotlieb-Stematsky.** 1988. Use of polyclonal and monoclonal antibodies and analysis of viral RNA in the detection of unusual group A human rotaviruses. J. Med. Virol. **25**:351–359.
2. **Air, G. M., W. G. Laver, and R. G. Webster.** 1987. Antigenic variation in influenza viruses. Contrib. Microbiol. Immunol. **8**:20–59.
3. **Albert, M. J., L. E. Unicomb, S. R. Tzipori, and R. F. Bishop.** 1987. Isolation and serotyping of animal rotaviruses and antigenic comparison with human rotaviruses. Arch. Virol. **93**: 123–130.
4. **Allen, A. M., and U. Desselberger.** 1985. Reassortment of human rotaviruses carrying rearranged genomes with bovine rotavirus. J. Gen. Virol. **66**:2703–2714.
5. **Altenburg, B. C., D. Y. Graham, and M. K. Estes.** 1980. Ultrastructural study of rotavirus replication in cultured cells. J. Gen. Virol. **46**:75–85.
6. **Andrew, M. E., D. B. Boyle, B. E. H. Coupar, P. L. Whitfeld, G. W. Both, and A. R. Bellamy.** 1987. Vaccinia virus recombinants expressing the SA11 rotavirus VP7 glycoprotein gene induce serotype-specific neutralizing antibodies. J. Virol. **61**: 1054–1060.
7. **Antczak, J. B., R. Chmelo, D. J. Pickup, and W. K. Joklik.** 1982. Sequences at both termini of the 10 genes of reovirus serotype 3 (strain Dearing). Virology **121**:307–319.
8. **Arias, C. F., T. Ballado, and M. Plebanski.** 1986. Synthesis of the outer-capsid glycoprotein of the simian rotavirus SA11 in *Escherichia coli*. Gene **47**:211–219.
9. **Arias, C. F., M. Lizano, and S. Lopez.** 1987. Synthesis in *Escherichia coli* and immunological characterization of a polypeptide containing the cleavage sites associated with trypsin enhancement of rotavirus SA11 infectivity. J. Gen. Virol. **68**:633–642.
10. **Arias, C. F., S. Lopez, J. R. Bell, and J. H. Strauss.** 1984. Primary structure of the neutralization antigen of simian rotavirus SA11 as deduced from cDNA sequence. J. Virol. **50**: 657–661.
11. **Arias, C. F., S. Lopez, and R. T. Espejo.** 1982. Gene protein products of SA11 simian rotavirus genome. J. Virol. **41**:42–50.
12. **Atkinson, P. H., and J. T. Lee.** 1984. Co-translational excision of α-glucose and α-mannose in nascent vesicular stomatitis virus G protein. J. Cell Biol. **98**:2245–2249.
13. **Au, K.-S., W.-K. Chan, J. W. Burns, and M. K. Estes.** 1989. Receptor activity of rotavirus nonstructural glycoprotein NS28. J. Virol. **63**:4553–4562.
14. **Au, K.-S., W.-K. Chan, and M. K. Estes.** 1989. Rotavirus morphogenesis involves an endoplasmic reticulum transmembrane glycoprotein, p. 257–267. *In* R. Compans, A. Helenius, and M. Oldstone (ed.), Cell biology of virus entry, replication and pathogenesis. Alan R. Liss, Inc., New York.
15. **Babiuk, L. A., and V. Misra.** 1979. Persistent infection of BSC-1 cells with rotavirus, p. 91–94. *In* F. Bricout, T. H. Flewett, M. Pensaert, and R. Scherrer (ed.), Viral enteritis in humans and animals. Institut National de la Santé et de la Recherche Médicale, Paris.
16. **Bartlett, A. V., A. J. Bednarz-Prashad, H. L. DuPont, and L. K. Pickering.** 1987. Rotavirus gastroenteritis. Annu. Rev. Med. **38**:399–415.
17. **Bartlett, A. V., R. R. Reves, and L. K. Pickering.** 1988. Rotavirus in infant-toddler day care centers; epidemiology relevant to disease control strategies. J. Pediatr. **113**:435–441.
18. **Bassel-Duby, R., D. R. Spriggs, K. L. Tyler, and B. N. Fields.** 1986. Identification of attenuating mutations on the reovirus type 3 S1 double-stranded RNA segment with a rapid sequencing technique. J. Virol. **60**:64–67.
19. **Bastardo, J. W., and I. H. Holmes.** 1980. Attachment of SA-11 rotavirus to erythrocyte receptors. Infect. Immun. **29**:1134–1140.
20. **Bastardo, J. W., J. L. McKimm-Breschkin, S. Sonza, L. D. Mercer, and I. H. Holmes.** 1981. Preparation and characterization of antisera to electrophoretically purified SA11 virus polypeptides. Infect. Immun. **34**:641–647.
21. **Baybutt, H. N., and M. A. McCrae.** 1984. The molecular biology of rotaviruses. VII. Detailed structural analysis of gene 10 of bovine rotavirus. Virus Res. **1**:533–541.
22. **Beards, G. M.** 1982. Polymorphism of genomic RNAs within rotavirus serotypes and subgroups. Arch. Virol. **74**:65–70.

22a. **Beards, G. M., and U. Desselberger.** 1989. Determination of rotavirus serotype-specific antibodies in sera by competitive enhanced enzyme immunoassay. J. Virol. Methods **24**:103–110.

22b. **Bellinzoni, R., X. Jiang, T. N. Tanaka, E. Scodeller, and M. K. Estes.** 1989. Rotavirus gene detection with biotinylated single-stranded RNA probes. Mol. Cell. Probes **3**:233–244.

22c. **Bellinzoni, R. C., J. Blackhall, N. Baro, N. Auza, N. Mattion, A. Casaro, J. L. La Torre, and E. A. Scodeller.** 1989. Efficacy of an inactivated oil-adjuvanted rotavirus vaccine in the control of calf diarrhoea in beef herds in Argentina. Vaccine **7**:263–268.

23. **Bellinzoni, R. C., N. M. Mattion, O. Burrone, A. Gonzalez, J. L. La Torre, and E. A. Scodeller.** 1987. Isolation of group A swine rotaviruses displaying atypical electropherotypes. J. Clin. Microbiol. **25**:952–954.

23a. **Bergmann, C. C., D. Maass, M. S. Poruchynsky, P. H. Atkinson, and R. Bellamy.** 1989. Topology of the non-structural rotavirus receptor glycoprotein NS28 in the rough endoplasmic reticulum. EMBO J. **8**:1695–1703.

24. **Besselaar, T. G., A. Rosenblatt, and A. H. Kidd.** 1986. Atypical rotavirus from South African neonates. Arch. Virol. **87**:327–330.
25. **Bican, P., J. Cohen, A. Charpilienne, and R. Scherrer.** 1982. Purification and characterization of bovine rotavirus cores. J. Virol. **43**:1113–1117.
26. **Birch, C. J., R. L. Heath, and I. D. Gust.** 1988. Use of serotype-specific monoclonal antibodies to study the epidemiology of rotavirus infection. J. Med. Virol. **24**:45–53.
27. **Biryahwaho, B., F. Hundley, and U. Desselberger.** 1987. Bovine rotavirus with rearranged genome reassorts with human rotavirus. Arch. Virol. **96**:257–264.
28. **Bishop, R. F., G. L. Barnes, E. Cipriani, and J. S. Lund.** 1983. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. N. Engl. J. Med. **309**:72–76.
29. **Boere, W. A. M., T. Harmsen, J. Vinje, B. J. Benaissa-Trouw, C. A. Kraaijeveld, and H. Snippe.** 1984. Identification of distinct antigenic determinants on Semliki Forest virus by using monoclonal antibodies with different antiviral activities. J. Virol. **52**:575–582.
30. **Borras-Cuesta, F., A. Petit-Camurdan, and Y. Fedon.** 1987. Engineering of immunogenic peptides by co-linear synthesis of determinants recognized by B and T cells. Eur. J. Immunol. **17**:1213–1215.
31. **Both, G. W., A. R. Bellamy, and L. J. Siegman.** 1984. Nucleotide sequence of the ds RNA genomic segment 7 of simian 11 rotavirus. Nucleic Acids Res. **12**:1621–1626.
32. **Both, G. W., A. R. Bellamy, J. E. Street, and L. J. Siegman.** 1982. A general strategy for cloning double-stranded RNA: nucleotide sequence of the simian-11 rotavirus gene 8. Nucleic Acids Res. **10**:7075–7088.
33. **Both, G. W., and P. A. Jennings.** 1987. Gene cloning to study viral antigens: prospects for novel influenza and rotavirus vaccines. Prog. Vet. Microbiol. Immunol. **3**:179–213.
34. **Both, G. W., J. S. Mattick, and A. R. Bellamy.** 1983. Serotype-specific glycoprotein of simian 11 rotavirus: coding assignment and gene sequence. Proc. Natl. Acad. Sci. USA **80**:3091–3095.
35. **Both, G. W., L. J. Siegman, A. R. Bellamy, and P. H. Atkinson.** 1983. Coding assignment and nucleotide sequence of simian

rotavirus SA11 gene segment 10: location of glycosylation sites suggests that the signal peptide is not cleaved. J. Virol. **48**:335–339.

36. **Both, G. W., L. J. Siegman, A. R. Bellamy, N. Ikegami, A. J. Shatkin, and Y. Furuichi.** 1984. Comparative sequence analysis of rotavirus genomic segment 6—the gene specifying viral subgroups 1 and 2. J. Virol. **51**:97–101.
37. **Boyle, J. F., and K. V. Holmes.** 1986. RNA-binding proteins of bovine rotavirus. J. Virol. **58**:561–568.
38. **Bremont, M., A. Charpilienne, D. Chabanne, and J. Cohen.** 1987. Nucleotide sequence and expression in *Escherichia coli* of the gene encoding the nonstructural protein NCVP2 of bovine rotavirus. Virology **161**:138–144.
39. **Bremont, M., J. Cohen, and M. A. McCrae.** 1988. Analysis of the structural polypeptides of a porcine group C rotavirus. J. Virol. **62**:2183–2185.

39a. **Bridger, J. C.** 1987. Novel rotaviruses in animals and man. CIBA Found. Symp. **128**:6–23.

40. **Bridger, J. C., and G. Oldham.** 1987. Avirulent rotavirus infections protect calves from disease with and without inducing high levels of neutralizing antibody. J. Gen. Virol. **68**: 2311–2317.

40a. **Bridger, J. C., S. Pedley, and M. A. McCrae.** 1986. Group C rotaviruses in humans. J. Clin. Microbiol. **23**:760–763.

40b. **Brown, D. W. G., G. M. Beards, G.-M. Chen, and T. H. Flewett.** 1987. Prevalence of antibody to group B (atypical) rotavirus in humans and animals. J. Clin. Microbiol. **25**:316–319.

41. **Brown, D. W. G., M. M. Mathan, M. Mathew, R. Martin, G. M. Beards, and V. I. Mathan.** 1988. Rotavirus epidemiology in Vellore, South India: group, subgroup, serotype, and electropherotype. J. Clin. Microbiol. **26**:2410–2414.
42. **Brussow, H., S. Marc-Martin, W. Eichhorn, J. Sidoti, and V. Fryder.** 1987. Characterization of a second bovine rotavirus serotype. Arch. Virol. **94**:29–41.
43. **Brussow, H., I. Walther, V. Fryder, J. Sidoti, and A. Bruttin.** 1988. Cross-neutralizing antibodies induced by single serotype vaccination of cows with rotavirus. J. Gen. Virol. **69**:1647–1658.
44. **Burns, J. W., D. Chen, M. K. Estes, and R. F. Ramig.** 1989. Biological and immunological characterization of a simian rotavirus SA11 variant with an altered genome segment 4. Virology **169**:427–435.
45. **Burns, J. W., H. B. Greenberg, R. D. Shaw, and M. K. Estes.** 1988. Functional and topographical analyses of epitopes on the hemagglutinin (VP4) of the simian rotavirus SA11. J. Virol. **62**:2164–2172.
46. **Burns, J. W., S. K. W. Welch, S. Nakata, and M. K. Estes.** 1989. Characterization of monoclonal antibodies to human group B rotavirus and their use in an antigen-detection enzyme-linked immunosorbent assay. J. Clin. Microbiol. **27**: 245–250.
47. **Caust, J., M. L. Dyall-Smith, I. Lazdins, and I. H. Holmes.** 1987. Glycosylation, an important modifier of rotavirus antigenicity. Arch. Virol. **96**:123–134.
48. **Chan, W.-K., K.-S. Au, and M. K. Estes.** 1988. Topography of the simian rotavirus nonstructural glycoprotein (NS28) in the endoplasmic reticulum membrane. Virology **164**:435–442.
49. **Chan, W.-K., M. E. Penaranda, S. E. Crawford, and M. K. Estes.** 1986. Two glycoproteins are produced from the rotavirus neutralization gene. Virology **151**:243–253.
50. **Chanas, A. C., D. S. Ellis, S. Stamford, and E. A. Gould.** 1982. Interaction of monoclonal antibodies directed against envelope glycoprotein E_1 of Sindbis virus with virus infected cells. Antiviral Res. **2**:191–201.
51. **Chanas, A. C., E. A. Gould, J. C. S. Clegg, and M. G. R. Varma.** 1982. Monoclonal antibodies to Sindbis virus glycoprotein E_1 can neutralize, enhance infectivity and independently inhibit haemagglutination or hemolysis. J. Gen. Virol. **58**:37–46.
52. **Chanock, R. M., B. R. Murphy, P. L. Collins, K. V. W. Coelingh, R. A. Olmsted, M. H. Snyder, M. K. Spriggs, G. A. Prince, B. Moss, J. Flores, M. Gorziglia, and A. Z. Kapikian.** 1988. Live viral vaccines for respiratory and enteric tract diseases. Vaccine **6**:129–133.
53. **Chanock, S. J., E. A. Wenske, and B. N. Fields.** 1983. Human rotaviruses and genomic RNA. J. Infect. Dis. **148**:49–50.
54. **Charpilienne, A., F. Borras, L. D'Auriol, F. Galibert, and J. Cohen.** 1986. Sequence of the gene encoding the outer glycoprotein of the bovine rotavirus (RF strain) and comparison with homologous genes from four bovine, simian and human rotaviruses. Ann. Inst. Pasteur/Virol. **137E**:71–77.
55. **Chasey, D.** 1977. Different particle types in tissue culture and intestinal epithelium infected with rotavirus. J. Gen. Virol. **37**:443–451.
56. **Chen, D., J. W. Burns, M. K. Estes, and R. F. Ramig.** 1989. The phenotypes of rotavirus reassortants depend upon the recipient genetic background. Proc. Natl. Acad. Sci. USA **86**:3743–3747.

56a. **Chen, G.-M., T. Hung, J. C. Bridger, and M. A. McCrae.** 1985. Chinese adult rotavirus is a group B rotavirus. Lancet **ii**:1123–1124.

57. **Chiarini, A., S. Arista, A. Giammanco, and A. Sinatra.** 1983. Rotavirus persistence in cell cultures: selection of resistant cells in the presence of foetal calf serum. J. Gen. Virol. **64**:1101–1110.
58. **Chiba, S., T. Yokoyama, S. Nakata, Y. Morita, T. Urasawa, K. Taniguchi, S. Urasawa, and T. Nakao.** 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. Lancet **ii**:417–421.
59. **Clark, B., and U. Desselberger.** 1988. Myristylation of rotavirus proteins. J. Gen. Virol. **69**:2681–2686.
60. **Clark, H. F., F. E. Borian, L. M. Bell, K. Modesto, V. Gouvea, and S. A. Plotkin.** 1988. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J. Infect. Dis. **158**:570–587.
61. **Clark, H. F., K. T. Dolan, P. Horton-Slight, J. Palmer, and S. A. Plotkin.** 1985. Diverse serologic response to rotavirus infection of infants in a single epidemic. Ped. Infect. Dis. J. **4**:626–631.
62. **Clark, H. F., Y. Hoshino, L. M. Bell, J. Groff, G. Hess, P. Bachman, and P. A. Offit.** 1987. Rotavirus isolate WI61 representing a presumptive new human serotype. J. Clin. Microbiol. **25**:1757–1762.
63. **Clark, S. M., B. B. Barnett, and R. S. Spendlove.** 1979. Production of high-titer bovine rotavirus with trypsin. J. Clin. Microbiol. **9**:413–417.
64. **Clark, S. M., J. R. Roth, M. L. Clark, B. B. Barnett, and R. S. Spendlove.** 1981. Trypsin enhancement of rotavirus infectivity: mechanism of enhancement. J. Virol. **39**:816–822.
65. **Clark, S. M., R. S. Spendlove, and B. B. Barnett.** 1980. Role of two particle types in bovine rotavirus morphogenesis. J. Virol. **34**:272–276.
66. **Cohen, J.** 1977. Ribonucleic acid polymerase activity associated with purified calf rotavirus. J. Gen. Virol. **36**:395–402.

66a. **Cohen, J., A. Charpilienne, S. Chilmonczyk, and M. K. Estes.** 1989. Nucleotide sequence of bovine rotavirus gene 1 and expression of the gene product in baculovirus. Virology **171**: 131–140.

67. **Cohen, J., J. Laporte, A. Charpilienne, and R. Scherrer.** 1979. Activation of rotavirus RNA polymerase by calcium chelation. Arch. Virol. **60**:177–186.
68. **Cohen, J., F. Lefevre, M. K. Estes, and M. Bremont.** 1984. Cloning of bovine rotavirus (RF strain): nucleotide sequence of the gene coding for the major capsid protein. Virology **138**: 178–182.
69. **Conner, M. E., M. K. Estes, and D. Y. Graham.** 1988. Rabbit model of rotavirus infection. J. Virol. **62**:1625–1633.
70. **Coulson, B. S.** 1987. Variation in neutralization epitopes of human rotaviruses in relation to genomic RNA polymorphism. Virology **159**:209–216.
71. **Coulson, B. S., K. J. Fowler, R. F. Bishop, and R. G. H. Cotton.** 1985. Neutralizing monoclonal antibodies to human rotavirus and indications of antigenic drift among strains from neonates. J. Virol. **54**:14–20.
72. **Coulson, B. S., K. J. Fowler, J. R. White, and R. G. H. Cotton.**

1987. Non-neutralizing monoclonal antibodies to a trypsin-sensitive site on the major glycoprotein of rotavirus which discriminate between virus serotypes. Arch. Virol. **93**:199–121.
73. **Coulson, B. S., J. M. Tursi, W. J. McAdam, and R. F. Bishop.** 1986. Derivation of neutralizing monoclonal antibodies to human rotaviruses and evidence that an immunodominant neutralization site is shared between serotypes 1 and 3. Virology **154**:301–312.
74. **Coulson, B. S., L. E. Unicomb, G. A. Pitson, and R. F. Bishop.** 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotaviruses. J. Clin. Microbiol. **25**:509–515.
75. **Cukor, G., and N. R. Blacklow.** 1984. Human viral gastroenteritis. Microbiol. Rev. **48**:157–179.
76. **Dharakul, T., M. Riepenhoff-Talty, B. Albini, and P. L. Ogra.** 1988. Distribution of rotavirus antigen in intestinal lymphoid tissues: potential development of the mucosal immune response to rotavirus. Clin. Exp. Immunol. **74**:14–19.
77. **di Matteo, A., A. Sarasini, M. S. Scotta, M. Parea, G. Licardi, and G. Gerna.** 1989. Nosocomial outbreak of infant rotavirus diarrhea due to the appearance of a new serotype 4 strain. J. Med. Virol. **27**:100–104.
78. **Dubois-Dalcq, M., K. V. Holmes, and B. Rentier (ed.).** 1984. Assembly of enveloped RNA viruses. Springer-Verlag, New York.
79. **Dyall-Smith, M. L., A. A. Azad, and I. H. Holmes.** 1983. Gene mapping of rotavirus double-stranded RNA segments by Northern blot hybridization: application to segments 7, 8, and 9. J. Virol. **46**:317–320.
80. **Dyall-Smith, M. L., T. C. Elleman, P. A. Hoyne, I. H. Holmes, and A. A. Azad.** 1983. Cloning and sequence of UK bovine rotavirus gene segment 7: marked sequence homology with simian rotavirus gene segment 8. Nucleic Acids Res. **11**: 3351–3362.
81. **Dyall-Smith, M. L., and I. H. Holmes.** 1981. Gene-coding assignments of rotavirus double-stranded RNA segments 10 and 11. J. Virol. **38**:1099–1103.
82. **Dyall-Smith, M. L., and I. H. Holmes.** 1981. Comparisons of rotavirus polypeptides by limited proteolysis: close similarity of certain polypeptides of different strains. J. Virol. **40**:720–728.
83. **Dyall-Smith, M. L., and I. H. Holmes.** 1984. Sequence homology between human and animal rotavirus serotype-specific glycoproteins. Nucleic Acids Res. **12**:3973–3982.
84. **Dyall-Smith, M. L., I. Lazdins, G. W. Tregear, and I. H. Holmes.** 1986. Location of the major antigenic sites involved in rotavirus serotype-specific neutralization. Proc. Natl. Acad. Sci. USA **83**:3465–3468.
85. **Eaton, B. T., and A. R. Gould.** 1987. Isolation and characterization of orbivirus genotypic variants. Virus Res. **6**:363–382.
86. **Edelman, R.** 1987. Perspective on the development and deployment of rotavirus vaccines. Pediatr. Infect. Dis. J. **6**:704–710.
87. **Elleman, T. C., P. A. Hoyne, M. L. Dyall-Smith, I. H. Holmes, and A. A. Azad.** 1983. Nucleotide sequence of the gene encoding the serotype-specific glycoprotein of UK bovine rotavirus. Nucleic Acids Res. **11**:4689–4701.
88. **Ericson, B. L., D. Y. Graham, B. B. Mason, and M. K. Estes.** 1982. Identification, synthesis, and modifications of simian rotavirus SA11 polypeptides in infected cells. J. Virol. **42**: 825–839.
89. **Ericson, B. L., D. Y. Graham, B. B. Mason, H. H. Hanssen, and M. K. Estes.** 1983. Two types of glycoprotein precursors are produced by the simian rotavirus SA11. Virology **127**:320–332.
90. **Esparza, J., and F. Gil.** 1978. A study on the ultrastructure of human rotavirus. Virology **91**:141–150.
91. **Espejo, R. T., S. Lopez, and C. F. Arias.** 1981. Structural polypeptides of simian rotavirus SA11 and the effect of trypsin. J. Virol. **37**:156–160.
92. **Espejo, R. T., F. Puerto, C. Soler, and N. Gonzalez.** 1984. Characterization of a human pararotavirus. Infect. Immun. **44**:112–116.
93. **Estes, M. K., M. E. Conner, M. A. Gilger, and D. Y. Graham.** 1989. Molecular biology and immunology of rotavirus infections. Immunol. Invest. **18**:571–581.
94. **Estes, M. K., S. E. Crawford, M. E. Penaranda, B. L. Petrie, J. W. Burns, W.-K. Chan, B. Ericson, G. E. Smith, and M. D. Summers.** 1987. Synthesis and immunogenicity of the rotavirus major capsid antigen using a baculovirus expression system. J. Virol. **61**:1488–1494.
95. **Estes, M. K., and D. Y. Graham.** 1985. Rotavirus antigens. Adv. Exp. Med. Biol. **185**:201–214.
96. **Estes, M. K., D. Y. Graham, and D. H. Dimitrov.** 1984. The molecular epidemiology of rotavirus gastroenteritis, p. 1–22. *In* J. L. Melnick (ed.), Progress in medical virology. S. Karger, Basel.
97. **Estes, M. K., D. Y. Graham, and B. B. Mason.** 1981. Proteolytic enhancement of rotavirus infectivity: molecular mechanisms. J. Virol. **39**:879–888.
98. **Estes, M. K., D. Y. Graham, E. M. Smith, and C. P. Gerba.** 1979. Rotavirus stability and inactivation. J. Gen. Virol. **43**: 403–409.
99. **Estes, M. K., B. B. Mason, S. Crawford, and J. Cohen.** 1984. Cloning and nucleotide sequence of the simian rotavirus gene 6 that codes for the major inner capsid protein. Nucleic Acids Res. **12**:1875–1887.
100. **Estes, M. K., E. L. Palmer, and J. F. Obijeski.** 1983. Rotaviruses: a review. Curr. Top. Microbiol. Immunol. **105**:123–184.
101. **Estes, M. K., and T. Tanaka.** 1989. Nucleic acid probes for rotavirus detection and characterization, p. 79–100. *In* F. Tenover (ed.), DNA probes for infectious diseases. CRC Press, Inc., Boca Raton, Fla.
102. **Evans, R. M., and S. M. Hollenberg.** 1988. Zinc fingers: gilt by association. Cell **52**:1–3.
103. **Fang, Z.-Y., R. I. Glass, M. Penaranda, H. Dong, S. S. Monroe, L. Wen, M. K. Estes, J. Eiden, R. H. Yolken, L. Saif, V. Gouvea, and T. Hung.** 1989. Purification and characterization of adult diarrhea rotavirus: identification of viral structural proteins. J. Virol. **63**:2191–2197.
104. **Farquhar, M. G., and G. E. Palade.** 1981. The Golgi apparatus (complex)—(1954–1981)—from artifact to center stage. J. Cell Biol. **91**:77s–103s.
105. **Faulkner-Valle, G. P., A. V. Clayton, and M. A. McCrae.** 1982. Molecular biology of rotaviruses. III. Isolation and characterization of temperature-sensitive mutants of bovine rotavirus. J. Virol. **42**:669–677.
106. **Flewett, T. H., A. S. Bryden, H. Davies, G. N. Woode, J. C. Bridger, and J. M. Derrick.** 1974. Relation between viruses from acute gastroenteritis of children and newborn calves. Lancet **ii**:61–63.
107. **Flewett, T. H., and G. N. Woode.** 1978. The rotaviruses. Brief review. Arch. Virol. **57**:1–23.
108. **Flores, J., K. Midthun, Y. Hoshino, K. Green, M. Gorziglia, A. Z. Kapikian, and R. M. Chanock.** 1986. Conservation of the fourth gene among rotaviruses recovered from asymptomatic newborn infants and its possible role in attenuation. J. Virol. **60**:972–979.
109. **Flores, J., I. Perez-Schael, M. Blanco, M. Vilar, D. Garcia, M. Perez, N. Daoud, K. Midthun, and A. Z. Kapikian.** 1989. Reactions to and antigenicity of two human-rhesus rotavirus reassortant vaccine candidates of serotypes 1 and 2 in Venezuelan infants. J. Clin. Microbiol. **27**:512–518.
110. **Francavilla, M., P. Miranda, A. DiMatteo, A. Sarasini, G. Gerna, and G. Milanesi.** 1987. Expression of bovine rotavirus neutralization antigen in *Escherichia coli*. J. Gen. Virol. **68**: 2975–2980.
111. **Frenchick, P., M. I. Sabara, M. K. Ijaz, and L. A. Babiuk.** 1988. Immune responses to synthetic peptide vaccines of veterinary importance, p. 141–151. *In* E. Kurstak, R. G. Marusyk, F. A. Murphy, and M. H. V. Van Regenmortel (ed.), Applied virology research, vol. 1. New vaccines and chemotherapy. Plenum Publishing Corp., New York.
112. **Friedman, M. G., A. Galil, B. Sarov, M. Margalith, G. Katzir, K. Midthun, K. Taniguchi, S. Urasawa, A. Z. Kapikian, R. Edelman, and I. Sarov.** 1988. Two sequential outbreaks of rotavirus gastroenteritis: evidence for symptomatic and asymptomatic reinfections. J. Infect. Dis. **158**:814–822.

112a. **Fukudome, K., O. Yoshie, and T. Konno.** 1989. Comparison of human, simian, and bovine rotaviruses for requirement of sialic acid in hemagglutination and cell adsorption. Virology **172**:196–205.

113. **Fukuhara, N., O. Yoshie, S. Kitaoka, and T. Konno.** 1988. Role of VP3 in human rotavirus internalization after target cell attachment via VP7. J. Virol. **62**:2209–2218.

114. **Fukuhara, N., O. Yoshie, S. Kitaoka, T. Konno, and N. Ishida.** 1987. Evidence for endocytosis-independent infection by human rotavirus. Arch. Virol. **97**:93–100.

115. **Furlong, D. B., M. L. Nibert, and B. N. Fields.** 1988. Sigma 1 protein of mammalian reoviruses extends from the surfaces of viral particles. J. Virol. **62**:246–256.

116. **Furuichi, Y., M. Morgan, S. Muthukrishnan, and A. J. Shatkin.** 1975. Reovirus messenger RNA contains a methylated, blocked 5′-terminal structure: $m^7G(5')ppp(5')G^mpCp$-. Proc. Natl. Acad. Sci. USA **72**:362–366.

117. **Garbarg-Chenon, A., F. Bricout, and J.-C. Nicolas.** 1986. Serological characterization of human reassortant rotaviruses. J. Virol. **59**:510–513.

118. **Garoff, H., A.-M. Frischauf, K. Simons, H. Lehrach, and H. Delius.** 1980. Nucleotide sequence of cDNA coding for Semliki Forest virus membrane glycoproteins. Nature (London) **288**: 236–241.

119. **Gary, G. W., Jr., D. R. Black, and E. Palmer.** 1982. Monoclonal IgG to the inner capsid of human rotavirus. Brief report. Arch. Virol. **72**:223–227.

120. **Gerna, G., A. Sarasini, A. di Matteo, M. Parea, P. Orsolini, and M. Battaglia.** 1988. Identification of two subtypes of serotype 4 human rotavirus by using VP7-specific neutralizing monoclonal antibodies. J. Clin. Microbiol. **26**:1388–1392.

121. **Gerna, G., A. Sarasini, A. di Matteo, N. Passarani, V. Gagliardi, G. Milanesi, G. C. B. A. Ricotti, and M. Battaglia.** 1988. The outer capsid glycoprotein VP7 of simian rotavirus SA11 contains two distinct neutralization epitopes. J. Gen. Virol. **69**:937–944.

122. **Glass, R. I., J. Keith, O. Nakagomi, T. Nakagomi, J. Askaa, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1985. Nucleotide sequence of the structural glycoprotein VP7 gene of Nebraska calf diarrhea virus rotavirus: comparison with homologous genes from four strains of human and animal rotaviruses. Virology **141**:292–298.

123. **Gombold, J. L., and R. F. Ramig.** 1986. Analysis of reassortment of genome segments in mice mixedly infected with rotaviruses SA11 and RRV. J. Virol. **57**:110–116.

124. **Gombold, J. L., and R. F. Ramig.** 1987. Assignment of simian rotavirus SA11 temperature-sensitive mutant groups A, C, F, and G to genome segments. Virology **161**:463–473.

124a. **González, S. A., N. M. Mattion, R. Bellinzoni, and O. R. Burrone.** 1989. Structure of rearranged genome segment 11 in two different rotavirus strains generated by a similar mechanism. J. Gen. Virol. **70**:1329–1336.

125. **Gorziglia, M., Y. Aguirre, Y. Hoshino, J. Esparza, I. Blumental, J. Askaa, M. Thompson, R. I. Glass, A. Z. Kapikian, and R. M. Chanock.** 1986. VP7 serotype-specific glycoprotein of OSU porcine rotavirus: coding assignment and gene sequence. J. Gen. Virol. **67**:2445–2454.

126. **Gorziglia, M., K. Green, K. Nishikawa, K. Taniguchi, R. Jones, A. Z. Kapikian, and R. M. Chanock.** 1988. Sequence of the fourth gene of human rotaviruses recovered from asymptomatic or symptomatic infections. J. Virol. **62**:2978–2984.

127. **Gorziglia, M., Y. Hoshino, A. Buckler-White, I. Blumentals, R. Glass, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1986. Conservation of amino acid sequence of VP8 and cleavage region of 84-kDa outer capsid protein among rotaviruses recovered from asymptomatic neonatal infection. Proc. Natl. Acad. Sci. USA **83**:7039–7043.

128. **Gorziglia, M., Y. Hoshino, K. Nishikawa, W. L. Maloy, R. W. Jones, A. Z. Kapikian, and R. M. Chanock.** 1988. Comparative sequence analysis of the genomic segment 6 of four rotaviruses each with a different subgroup specificity. J. Gen. Virol. **69**:1659–1669.

129. **Gorziglia, M., C. Lanea, F. Liprandi, and J. Esparza.** 1985. Biochemical evidence for the oligomeric (possibly trimeric) structure of the major inner capsid polypeptide (45K) of rotavirus. J. Gen. Virol. **66**:1889–1900.

129a. **Gorziglia, M., K. Nishikawa, and N. Fukuhara.** 1989. Evidence of duplication and deletion in super short segment 11 of rabbit rotavirus Alabama strain. Virology **170**:587–590.

130. **Gorziglia, M., K. Nishikawa, K. Green, and K. Taniguchi.** 1988. Gene sequence of the VP7 serotype specific glycoprotein of Gottfried porcine rotavirus. Nucleic Acids Res. **16**:775.

131. **Graham, A., G. Kudesia, A. M. Allen, and U. Desselberger.** 1987. Reassortment of human rotavirus possessing genome rearrangements with bovine rotavirus: evidence for host cell selection. J. Gen. Virol. **68**:115–122.

132. **Graham, D. Y., and M. K. Estes.** 1980. Proteolytic enhancement of rotavirus infectivity: biologic mechanisms. Virology **101**:432–439.

133. **Graham, D. Y., and M. K. Estes.** 1985. Proposed working serologic classification system for rotaviruses. Ann. Inst. Pasteur/Virol. **136E**:5–12.

134. **Green, K. Y., Y. Hoshino, and N. Ikegami.** 1989. Sequence analysis of the gene encoding the serotype-specific glycoprotein (VP7) of two new human rotavirus serotypes. Virology **168**:429–433.

135. **Green, K. Y., K. Midthun, M. Gorziglia, Y. Hoshino, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1987. Comparison of the amino acid sequences of the major neutralization protein of four human rotavirus serotypes. Virology **161**:153–159.

136. **Green, K. Y., J. F. Sears, K. Taniguchi, K. Midthun, Y. Hoshino, M. Gorziglia, K. Nishikawa, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1988. Prediction of human rotavirus serotype by nucleotide sequence analysis of the VP7 protein gene. J. Virol. **62**:1819–1823.

137. **Greenberg, H. B., J. Flores, A. R. Kalica, R. G. Wyatt, and R. Jones.** 1983. Gene coding assignments for growth restriction, neutralization and subgroup specificities of the W and DS-1 strains of human rotavirus. J. Gen. Virol. **64**:313–320.

138. **Greenberg, H. B., A. R. Kalica, R. W. Wyatt, R. D. Jones, A. Z. Kapikian, and R. M. Chanock.** 1981. Rescue of noncultivatable human rotavirus by gene reassortment during mixed infection with ts mutants of a cultivatable bovine rotavirus. Proc. Natl. Acad. Sci. USA **78**:420–424.

139. **Greenberg, H. B., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh.** 1982. Serological analysis of the subgroup protein of rotavirus, using monoclonal antibodies. Infect. Immun. **39**:91–99.

139a. **Greenberg, H. B., J. Valdesuso, K. van Wyke, K. Midthun, M. Walsh, V. McAuliffe, R. G. Wyatt, A. R. Kalica, J. Flores, and Y. Hoshino.** 1983. Production and preliminary characterization of monoclonal antibodies directed at two surface proteins of rhesus rotavirus. J. Virol. **47**:267–275.

140. **Greenberg, H. B., P. T. Vo, and R. Jones.** 1986. Cultivation and characterization of three strains of murine rotavirus. J. Virol. **57**:585–590.

141. **Grimwood, K., J. C. S. Lund, B. S. Coulson, I. L. Hudson, R. F. Bishop, and G. L. Barnes.** 1988. Comparison of serum and mucosal antibody responses following severe acute rotavirus gastroenteritis in young children. J. Clin. Microbiol. **26**:732–738.

142. **Gunn, P. R., F. Sato, K. F. H. Powell, A. R. Bellamy, J. R. Napier, D. R. K. Harding, W. S. Hancock, L. J. Siegman, and G. W. Both.** 1985. Rotavirus neutralizing protein VP7: antigenic determinants investigated by sequence analysis and peptide synthesis. J. Virol. **54**:791–797.

143. **Halsey, N. A., E. L. Anderson, S. D. Sears, M. Steinhoff, M. Wilson, R. B. Belshe, K. Midthun, A. Z. Kapikian, B. Burns, and M. L. Clements.** 1988. Human-rhesus reassortant rotavirus vaccines: safety and immunogenicity in adults, infants, and children. J. Infect. Dis. **158**:1261–1267.

144. **Heath, R., C. Birch, and I. Gust.** 1986. Antigenic analysis of rotavirus isolates using monoclonal antibodies specific for human serotypes 1, 2, 3 and 4, and SA11. J. Gen. Virol. **67**:2455–2466.

145. **Helmberger-Jones, M., and J. T. Patton.** 1986. Characteriza-

tion of subviral particles in cells infected with simian rotavirus SA11. Virology **155**:655–665.
146. **Hilpert, H., H. Brussow, C. Mietens, J. Sidoti, L. Lerner, and H. Werchau.** 1987. Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. J. Infect. Dis. **156**:158–166.
147. **Ho, M.-S., R. I. Glass, P. F. Pinsky, and L. J. Anderson.** 1988. Rotavirus as a cause of diarrheal morbidity and mortality in the United States. J. Infect. Dis. **158**:1112–1116.
148. **Hofer, J. M. I., F. Sato, J. E. Street, and A. R. Bellamy.** 1987. Nucleotide sequence for gene 6 of rotavirus strain S2. Nucleic Acids Res. **15**:7175.
149. **Holmes, I. H.** 1983. Rotaviruses, p. 359–423. *In* W. K. Joklik (ed.), The Reoviridae. Plenum Publishing Corp., New York.
150. **Hoshino, Y., M. Gorziglia, J. Valdesuso, J. Askaa, R. I. Glass, and A. Z. Kapikian.** 1987. An equine rotavirus (FI-14) which bears both subgroup I and subgroup II specificities on its VP6. Virology **157**:488–496.
151. **Hoshino, Y., L. J. Saif, M. M. Sereno, R. M. Chanock, and A. Z. Kapikian.** 1988. Infection immunity of piglets to either VP3 or VP7 outer capsid protein confers resistance to challenge with a virulent rotavirus bearing the corresponding antigen. J. Virol. **62**:744–748.
152. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, R. M. Chanock, and A. Z. Kapikian.** 1987. Analysis by plaque reduction neutralization assay of intertypic rotaviruses suggests that gene reassortment occurs in vivo. J. Clin. Microbiol. **25**: 290–294.
153. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**:8701–8704.
154. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. J. Infect. Dis. **149**:694–702.
155. **Hrdy, D. B.** 1987. Epidemiology of rotaviral infection in adults. Rev. Infect. Dis. **9**:461–469.
156. **Hubbard, S. C., and R. J. Ivatt.** 1981. Synthesis and processing of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **50**:555–583.
157. **Hum, C. P., M. L. Dyall-Smith, and I. H. Holmes.** 1989. The VP7 gene of a new G serotype of human rotavirus (B37) is similar to G3 proteins in the antigenic C region. Virology **170**:55–61.
158. **Hundley, F., B. Biryahwaho, M. Gow, and U. Desselberger.** 1985. Genome rearrangements of bovine rotavirus after serial passage at high multiplicity of infection. Virology **143**:88–103.
159. **Hundley, F., M. McIntyre, B. Clark, G. Beards, D. Wood, I. Chrystie, and U. Desselberger.** 1987. Heterogeneity of genome rearrangements in rotaviruses isolated from a chronically infected immunodeficient child. J. Virol. **61**:3365–3372.
160. **Hung, T., C. Wang, Z. Fang, Z. Chou, X. Chang, X. Liong, G. Chen, H. Yao, T. Chao, W. Ye, S. Den, and W. Chang.** 1984. Waterborne outbreak of rotavirus diarrhea in adults in China caused by a novel rotavirus. Lancet **i**:1139–1142.
161. **Imai, M., K. Akatani, N. Ikegami, and Y. Furuichi.** 1983. Capped and conserved terminal structures in human rotavirus genome double-stranded RNA segments. J. Virol. **47**:125–136.
162. **Imai, M., M. A. Richardson, N. Ikegami, A. J. Shatkin, and Y. Furuichi.** 1982. Cloning and nucleotide sequencing of Reoviridae genomes, p. 15–26. *In* R. W. Compans and D. H. L. Bishop (ed.), Double-stranded RNA viruses. Elsevier Biomedical Press, New York.
163. **Imai, M., M. A. Richardson, N. Ikegami, A. J. Shatkin, and Y. Furuichi.** 1983. Molecular cloning of double-stranded RNA virus genomes. Proc. Natl. Acad. Sci. USA **80**:373–377.
164. **Johnson, M. A., and M. A. McCrae.** 1989. Molecular biology of rotaviruses. VIII. Quantitative analysis of regulation of gene expression during virus replication. J. Virol. **63**:2048–2055.
165. **Kabcenell, A. K., and P. H. Atkinson.** 1985. Processing of the rough endoplasmic reticulum membrane glycoproteins of rotavirus SA11. J. Cell Biol. **101**:1270–1280.
166. **Kabcenell, A. K., M. S. Poruchynsky, A. R. Bellamy, H. B. Greenberg, and P. H. Atkinson.** 1988. Two forms of VP7 are involved in assembly of SA11 rotavirus in endoplasmic reticulum. J. Virol. **62**:2929–2941.
167. **Kalica, A. R., J. Flores, and H. B. Greenberg.** 1983. Identification of the rotaviral gene that codes for the hemagglutinin and protease-enhanced plaque formation. Virology **125**:194–205.
168. **Kalica, A. R., H. B. Greenberg, R. G. Wyatt, J. Flores, M. M. Sereno, A. Z. Kapikian, and R. M. Chanock.** 1981. Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens. Virology **112**: 385–390.
169. **Kaljot, K. T., R. D. Shaw, D. H. Rubin, and H. B. Greenberg.** 1988. Infectious rotavirus enters cells by direct cell membrane penetration, not by endocytosis. J. Virol. **62**:1136–1144.
170. **Kantharidis, P., M. L. Dyall-Smith, and I. H. Holmes.** 1987. Marked sequence variation between segment 4 genes of human RV-5 and simian SA11 rotaviruses. Arch. Virol. **93**:111–121.
171. **Kantharidis, P., M. L. Dyall-Smith, G. W. Tregear, and I. H. Holmes.** 1988. Nucleotide sequence of UK bovine rotavirus segment 4: possible host restriction of VP3 genes. Virology **166**:308–315.
172. **Kapahnke, R., W. Rappold, U. Desselberger, and D. Riesner.** 1986. The stiffness of dsRNA: hydrodynamic studies on fluorescence-labelled RNA segments of bovine rotavirus. Nucleic Acids Res. **14**:3215–3228.
173. **Kapikian, A. Z., and R. M. Chanock.** 1985. Rotaviruses, p. 863–906. *In* B. N. Fields (ed.), Virology. Raven Press, New York.
174. **Kapikian, A. Z., W. L. Cline, H. B. Greenberg, R. G. Wyatt, A. R. Kalica, C. E. Banks, H. D. James, J. Flores, and R. M. Chanock.** 1981. Antigenic characterization of human and animal rotaviruses by immune adherence hemagglutination assay (IAHA): evidence for distinctness of IAHA and neutralization antigens. Infect. Immun. **33**:415–425.
175. **Kapikian, A. Z., J. Flores, Y. Hoshino, R. I. Glass, K. Midthun, M. Gorziglia, and R. M. Chanock.** 1986. Rotavirus: the major etiologic agent of severe infantile diarrhea may by controllable by a "Jennerian" approach to vaccination. J. Infect. Dis. **153**:815–822.
176. **Keljo, D. J., M. Kuhn, and A. Smith.** 1988. Acidification of endosomes is not important for the entry of rotavirus into the cell. J. Pediatr. Gastroenterol. Nutr. **7**:257–263.
177. **Keljo, D. J., and A. K. Smith.** 1988. Characterization of binding of simian rotavirus SA-11 to cultured epithelial cells. J. Pediatr. Gastroenterol. Nutr. **7**:249–256.
178. **Killen, H. M., and N. J. Dimmock.** 1982. Identification of a neutralization-specific antigen of a calf rotavirus. J. Gen. Virol. **62**:297–311.
179. **Kitaoka, S., N. Fukuhara, F. Tazawa, H. Suzuki, T. Sato, T. Konno, T. Ebino, and N. Ishida.** 1986. Characterization of monoclonal antibodies against human rotavirus hemagglutinin. J. Med. Virol. **19**:313–323.
180. **Kitaoka, S., T. Nakagomi, N. Fukuhara, Y. Hoshino, H. Suzuki, O. Nakagomi, A. Z. Kapikian, T. Ebina, T. Konno, and N. Ishida.** 1987. Serologic characteristics of human rotavirus isolate, AU-1, which has a "long" RNA pattern and subgroup I specificity. J. Med. Virol. **23**:351–357.
181. **Kogasaka, R., M. Akihara, K. Horino, S. Chiba, and T. Nakao.** 1979. A morphological study of human rotavirus. Arch. Virol. **61**:41–48.
182. **Kohl, S., M. W. Harmon, and Y. P. Tang.** 1983. Cytokine-stimulated human natural killer cytotoxicity: response to rotavirus-infected cells. Pediatr. Res. **17**:868–872.
183. **Kouvelos, K., M. Petric, and P. J. Middleton.** 1984. Oligosaccharide composition of calf rotavirus. J. Gen. Virol. **65**: 1159–1164.
184. **Kouvelos, K., M. Petric, and P. J. Middleton.** 1984. Comparison of bovine, simian and human rotavirus structural glycoproteins. J. Gen. Virol. **65**:1211–1214.
185. **Kozak, M.** 1981. Possible role of flanking nucleotides in recog-

nition of the AUG initiator codon by eukaryotic ribosomes. Nucleic Acids Res. **9**:5233–5250.
186. **Kozak, M.** 1986. Bifunctional messenger RNAs in eukaryotes. Cell **47**:481–483.
187. **Kuchino, Y., S. Nishimura, R. E. Smith, and Y. Furuichi.** 1982. Homologous terminal sequences in the double-stranded RNA genome segments of cytoplasmic polyhedrosis virus of the silkworm *Bombyx mori*. J. Virol. **44**:538–543.
188. **Kumar, A., A. Charpilienne, and J. Cohen.** 1989. Nucleotide sequence of the gene encoding for the RNA binding protein (VP2) of RF bovine rotavirus. Nucleic Acids Res. **17**:2126.
189. **Lambert, J.-P., P. Marbehant, D. Marissens, and G. Zissis.** 1984. Monoclonal antibodies directed against different antigenic determinants of rotavirus. J. Virol. **51**:47–51.
190. **Lambert, J.-P., D. Marissens, P. Marbehant, and G. Zissis.** 1983. Prevalence of subgroup 1, 2 and 3 rotaviruses in Belgian children suffering from acute diarrhea (1979–1981). J. Med. Virol. **11**:31–38.
191. **Lanata, C. F., R. E. Black, R. del Aguila, A. Gil, H. Verastegui, G. Gerna, J. Flores, A. Z. Kapikian, and F. E. Andre.** 1989. Protection of Peruvian children against rotavirus diarrhea of specific serotypes by one, two, or three doses of the RIT 4237 attenuated bovine rotavirus vaccine. J. Infect. Dis. **159**:452–459.
192. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1988. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science **240**:1759–1764.
192a. **Lazzarini, R. A., J. D. Keene, and M. Schubert.** 1981. The origins of defective interfering particles of the negative-strand RNA viruses. Cell **26**:145–154.
193. **Linhares, A. C., Y. B. Gabbay, J. D. P. Mascarenhas, R. B. Freitas, T. H. Flewett, and G. M. Beards.** 1988. Epidemiology of rotavirus subgroups and serotypes in Belem, Brazil: a three-year study. Ann. Inst. Pasteur/Virol. **139**:89–99.
194. **Liu, M., P. A. Offit, and M. K. Estes.** 1988. Identification of the simian rotavirus SA11 genome segment 3 product. Virology **163**:26–32.
195. **Lopez, S., and C. F. Arias.** 1987. The nucleotide sequence of the 5′ and 3′ ends of rotavirus SA11 gene 4. Nucleic Acids Res. **15**:4691.
196. **Lopez, S., C. F. Arias, J. R. Bell, J. H. Strauss, and R. T. Espejo.** 1985. Primary structure of the cleavage site associated with trypsin enhancement of rotavirus SA11 infectivity. Virology **144**:11–19.
197. **Lopez, S., C. F. Arias, E. Mendez, and R. T. Espejo.** 1986. Conservation in rotaviruses of the protein region containing the two sites associated with trypsin enhancement of infectivity. Virology **154**:224–227.
198. **Losonsky, G. A., J. P. Johnson, J. A. Winkelstein, and R. H. Yolken.** 1985. Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis: a pharmacokinetic and functional analysis. J. Clin. Invest. **76**:2362–2367.
199. **Losonsky, G. A., M. B. Rennels, Y. Lim, G. Krall, A. Z. Kapikian, and M. M. Levin.** 1988. Systemic and mucosal immune responses to rhesus rotavirus vaccine MMU 18006. Pediatr. Infect. Dis. J. **7**:388–393.
200. **Ludert, J. E., F. Gil, F. Liprandi, and J. Esparza.** 1986. The structure of the rotavirus inner capsid studied by electron microscopy of chemically disrupted particles. J. Gen. Virol. **67**:1721–1725.
201. **Ludert, J. E., F. Michelangeli, F. Gil, F. Liprandi, and J. Esparza.** 1987. Penetration and uncoating of rotaviruses in cultured cells. Intervirology **27**:95–101.
202. **Mackow, E. R., J. W. Barnett, H. Chan, and H. B. Greenberg.** 1989. The rhesus rotavirus outer capsid protein NP4 functions as a hemagglutinin and is antigenically conserved when expressed by a baculovirus recombinant. J. Virol. **63**:1661–1668.
203. **Mackow, E. R., R. D. Shaw, S. M. Matsui, P. T. Vo, D. A. Benfield, and H. B. Greenberg.** 1988. Characterization of homotypic and heterotypic VP7 neutralization sites of rhesus rotavirus. Virology **165**:511–517.
204. **Mackow, E. R., R. D. Shaw, S. M. Matsui, P. T. Vo, M. N. Dang, and H. B. Greenberg.** 1988. Characterization of the rhesus rotavirus VP3 gene: location of amino acids involved in homologous and heterologous rotavirus neutralization and identification of a putative fusion region. Proc. Natl. Acad. Sci. USA **85**:645–649.
204a. **Makino, S., J. G. Keck, S. A. Stohlman, and M. M. C. Lai.** 1986. High-frequency RNA recombination of murine coronaviruses. J. Virol. **57**:729–737.
205. **Martin, M. L., E. L. Palmer, and P. J. Middleton.** 1975. Ultrastructure of infantile gastroenteritis virus. Virology **68**:146–153.
206. **Mason, B. B., S. K. Dheer, C.-L. Hsiao, G. Zandle, B. Kostek, E. I. Rosanoff, P. P. Hung, and A. R. Davis.** 1985. Sequence of the serotype-specific glycoprotein of the human rotavirus Wa strain and comparison with other human rotavirus serotypes. Virus Res. **2**:291–299.
207. **Mason, B. B., D. Y. Graham, and M. K. Estes.** 1980. In vitro transcription and translation of simian rotavirus SA11 gene products. J. Virol. **33**:1111–1121.
208. **Mason, B. B., D. Y. Graham, and M. K. Estes.** 1983. Biochemical mapping of the simian rotavirus SA11 genome. J. Virol. **46**:413–423.
209. **Matsui, S. M., E. R. Mackow, and H. B. Greenberg.** 1989. Molecular determinants of rotavirus neutralization and protection. Adv. Virus Res. **36**:181–214.
210. **Matsui, S. M., P. A. Offit, P. T. Vo, E. R. Mackow, D. A. Benfield, R. D. Shaw, L. Padilla-Noriega, and H. B. Greenberg.** 1989. Passive protection against rotavirus-induced diarrhea by monoclonal antibodies to the heterotypic neutralization domain of VP7 and the VP8 fragment of VP4. J. Clin. Microbiol. **27**:780–782.
211. **Matsuno, S., A. Hasegawa, A. R. Kalica, and R. Kono.** 1980. Isolation of a recombinant between simian and bovine rotaviruses. J. Gen. Virol. **48**:253–256.
212. **Matsuno, S., A. Hasegawa, A. Mukoyama, and S. Inouye.** 1985. A candidate for a new serotype of human rotavirus. J. Virol. **54**:623–624.
213. **Matsuno, S., and S. Inouye.** 1983. Purification of an outer capsid glycoprotein in neonatal calf diarrhea virus and preparation of its antisera. Infect. Immun. **39**:155–158.
214. **Matsuno, S., A. Mukoyama, A. Hasegawa, K. Taniguchi, and S. Inouye.** 1988. Characterization of a human rotavirus strain which is possibly a naturally-occurring reassortant virus. Virus Res. **10**:167–175.
215. **Mattion, N., S. A. Gonzalez, O. Burrone, R. Bellinzoni, J. L. La Torre, and E. A. Scodeller.** 1988. Rearrangement of genomic segment 11 in two swine rotavirus strains. J. Gen. Virol. **69**:695–698.
216. **McCrae, M. A., and G. P. Faulkner-Valle.** 1981. Molecular biology of rotaviruses. I. Characterization of basic growth parameters and pattern of macromolecular synthesis. J. Virol. **39**:490–496.
217. **McCrae, M. A., and J. G. McCorquodale.** 1982. Molecular biology of rotaviruses. II. Identification of the protein-coding assignments of calf rotavirus genome RNA species. Virology **117**:435–443.
218. **McCrae, M. A., and J. G. McCorquodale.** 1983. Molecular biology of rotaviruses. V. Terminal structure of viral RNA species. Virology **126**:204–212.
219. **McCrae, M. A., and J. G. McCorquodale.** 1987. Expression of a major bovine rotavirus neutralization antigen (VP7c) in *Escherichia coli*. Gene **55**:9–18.
220. **McIntyre, M., V. Rosenbaum, W. Rappold, M. Desselberger, D. Wood, and U. Desselberger.** 1987. Biophysical characterization of rotavirus particles containing rearranged genomes. J. Gen. Virol. **68**:2961–2966.
221. **McNulty, M. S., W. L. Curran, and J. B. McFerran.** 1976. The morphogenesis of a cytopathic bovine rotavirus in Madin-Darby bovine kidney cells. J. Gen. Virol. **33**:503–508.
222. **Mertens, P. P. C., and D. V. Sangar.** 1985. Analysis of the terminal sequences of the genome segments of four orbiviruses, p. 371–387. *In* T. L. Barber and M. M. Jochim (ed.), Bluetongue and related orbiviruses. Alan R. Liss, Inc., New York.

222a. **Meyer, J. C., C. C. Bergmann, and A. R. Bellamy.** 1989. Interaction of rotavirus cores with the non-structural glycoprotein NS28. Virology **171**:98–107.
223. **Midthun, K., H. B. Greenberg, Y. Hoshino, A. Z. Kapikian, R. G. Wyatt, and R. M. Chanock.** 1985. Reassortant rotaviruses as potential live rotavirus vaccine candidates. J. Virol. **53**:949–954.
224. **Midthun, K., J. Valdesuso, Y. Hoshino, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1987. Analysis by RNA-RNA hybridization assay of intertypic rotaviruses suggests that gene reassortment occurs in vivo. J. Clin. Microbiol. **25**:295–300.
225. **Mitchell, D. B., and G. W. Both.** 1988. Simian rotavirus SA11 segment 11 contains overlapping reading frames. Nucleic Acids Res. **16**:6244.
225a. **Mitchell, D. B., and G. W. Both.** 1989. Complete nucleotide sequence of the simian rotavirus SA11 VP4 gene. Nucleic Acids Res. **17**:2122.
226. **Munro, S., and H. R. B. Pelham.** 1987. A C-terminal signal prevents secretion of luminal ER proteins. Cell **48**:899–907.
227. **Musalem, C., and R. T. Espejo.** 1985. Release of progeny virus from cells infected with simian rotavirus SA11. J. Gen. Virol. **66**:2715–2724.
228. **Nagesha, H. S., and I. H. Holmes.** 1988. New porcine rotavirus serotype antigenically related to human rotavirus serotype 3. J. Clin. Microbiol. **26**:171–174.
229. **Nakagomi, O., T. Nakagomi, Y. Hoshino, J. Flores, and A. Z. Kapikian.** 1987. Genetic analysis of a human rotavirus that belongs to subgroup I but has an RNA pattern typical of subgroup II human rotaviruses. J. Clin. Microbiol. **25**:1159–1164.
230. **Nakagomi, T., K. Akatani, N. Ikegami, N. Katsushima, and O. Nakagomi.** 1988. Occurrence of changes in human rotavirus serotypes with concurrent changes in genomic RNA electropherotypes. J. Clin. Microbiol. **26**:2586–2592.
231. **Nakata, S., M. K. Estes, D. Y. Graham, R. Loosle, T. Hung, S. Wang, L. J. Saif, and J. L. Melnick.** 1986. Human group B rotavirus: antigenic characterization and ELISA detection of adult diarrhea rotaviruses. J. Infect. Dis. **154**:448–455.
232. **Nakata, S., M. K. Estes, D. Y. Graham, S. Wang, G. W. Gary, and J. L. Melnick.** 1987. Detection of antibody to group B adult diarrhea rotaviruses in humans. J. Clin. Microbiol. **25**:812–818.
233. **Nakata, S., B. L. Petrie, E. P. Calomeni, and M. K. Estes.** 1987. Electron microscopy procedure influences detection of rotaviruses. J. Clin. Microbiol. **25**:1902–1906.
234. **Neurath, A. R., and S. B. H. Kent.** 1988. The pre-S region of hepadnavirus envelope proteins. Adv. Virus Res. **34**:65–142.
234a. **Nishikawa, K., Y. Hoshino, K. Taniguchi, K. Y. Green, H. B. Greenberg, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1989. Rotavirus VP7 neutralization epitopes of serotype 3 strains. Virology **171**:503–515.
235. **Nishikawa, K., K. Taniguchi, A. Torres, Y. Hoshino, K. Green, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1988. Comparative analysis of the VP3 gene of divergent strains of rotaviruses simian SA11 and bovine Nebraska calf diarrhea virus. J. Virol. **62**:4022–4026.
236. **Novo, E., and J. Esparza.** 1981. Composition and topography of structural polypeptides of bovine rotavirus. J. Gen. Virol. **56**:325–335.
236a. **Nuttall, S. D., C. P. Hum, I. H. Holmes, and M. L. Dyall-Smith.** 1989. Sequences of VP9 genes from short and supershort rotavirus strains. Virology **171**:453–457.
237. **Offit, P. A., and G. Blavat.** 1986. Identification of the two rotavirus genes determining neutralization specificities. J. Virol. **57**:376–378.
238. **Offit, P. A., G. Blavat, H. B. Greenberg, and H. F. Clark.** 1986. Molecular basis of rotavirus virulence: role of gene segment 4. J. Virol. **57**:46–49.
239. **Offit, P. A., H. F. Clark, G. Blavat, and H. B. Greenberg.** 1986. Reassortant rotaviruses containing structural proteins vp3 and vp7 from different parents induce antibodies protective against each parental serotype. J. Virol. **60**:491–496.
240. **Offit, P. A., H. F. Clark, and S. A. Plotkin.** 1983. Response of mice to rotaviruses of bovine or primate origin assessed by radioimmunoassay, radioimmunoprecipitation, and plaque reduction neutralization. Infect. Immun. **42**:293–300.
241. **Offit, P. A., H. F. Clark, A. H. Taylor, R. G. Hess, P. A. Bachmann, and S. A. Plotkin.** 1984. Rotavirus-specific antibodies in fetal bovine serum and commercial preparations of serum albumin. J. Clin. Microbiol. **20**:266–270.
242. **Offit, P. A., and K. I. Dudzik.** 1988. Rotavirus-specific cytotoxic T lymphocytes cross-react with target cells infected with different rotavirus serotypes. J. Virol. **62**:127–131.
243. **Offit, P. A., R. D. Shaw, and H. B. Greenberg.** 1986. Passive protection against rotavirus-induced diarrhea by monoclonal antibodies to surface proteins vp3 and vp7. J. Virol. **58**:700–703.
244. **Okada, Y., M. A. Richardson, N. Ikegami, A. Nomoto, and Y. Furuichi.** 1984. Nucleotide sequence of human rotavirus genome segment 10, an RNA encoding a glycosylated virus protein. J. Virol. **51**:856–859.
245. **Omar, A., and H. Koblet.** 1988. Semliki Forest virus particles containing only the E_1 envelope glycoprotein are infectious and can induce cell-cell fusion. Virology **166**:17–23.
246. **Patton, J. T.** 1986. Synthesis of simian rotavirus SA11 double-stranded RNA in a cell-free system. Virus Res. **6**:217–233.
247. **Patton, J. T., and C. O. Gallegos.** 1988. Structure and protein composition of the rotavirus replicase particle. Virology **166**:358–365.
248. **Patton, J. T., and S. Stacy-Phipps.** 1986. Electrophoretic separation of the plus and minus strands of rotavirus SA11 double-stranded RNAs. J. Virol. Methods **13**:185–190.
249. **Pedley, S., J. C. Bridger, J. F. Brown, and M. A. McCrae.** 1983. Molecular characterization of rotaviruses with distinct group antigens. J. Gen. Virol. **64**:2093–2101.
250. **Pedley, S., F. Hundley, I. Chrystie, M. A. McCrae, and U. Desselberger.** 1984. The genomes of rotaviruses isolated from chronically infected immunodeficient children. J. Gen. Virol. **65**:1141–1150.
251. **Persing, D. H., H. E. Varmus, and D. Ganem.** 1985. A frameshift mutation in the pre-S region of the human hepatitis B virus genome allows production of surface antigen particles but eliminates binding to polymerized albumin. Proc. Natl. Acad. Sci. USA **82**:3440–3444.
252. **Petrie, B. L., M. K. Estes, and D. Y. Graham.** 1983. Effects of tunicamycin on rotavirus morphogenesis and infectivity. J. Virol. **46**:270–274.
253. **Petrie, B. L., D. Y. Graham, and M. K. Estes.** 1981. Identification of rotavirus particle types. Intervirology **16**:20–28.
254. **Petrie, B. L., D. Y. Graham, H. Hanssen, and M. K. Estes.** 1982. Localization of rotavirus antigens in infected cells by ultrastructural immunocytochemistry. J. Gen. Virol. **63**:457–467.
255. **Petrie, B. L., H. B. Greenberg, D. Y. Graham, and M. K. Estes.** 1984. Ultrastructural localization of rotavirus antigens using colloidal gold. Virus Res. **1**:133–152.
256. **Pocock, D. H.** 1987. Isolation and characterization of two group A rotaviruses with unusual genome profiles. J. Gen. Virol. **68**:653–660.
257. **Poruchynsky, M. S., and P. H. Atkinson.** 1988. Primary sequence domains required for the retention of rotavirus VP7 in the endoplasmic reticulum. J. Cell Biol. **107**:1697–1706.
258. **Poruchynsky, M. S., A. K. Kabcenell, and P. H. Atkinson.** 1989. Sequence requirements for retention of rotavirus VP7 in the endoplasmic reticulum, p. 243–255. *In* R. Compans, A. Helenius, and M. Oldstone (ed.), Cell biology of virus entry, replication and pathogenesis. Alan R. Liss, Inc., New York.
259. **Poruchynsky, M. S., C. Tyndall, G. W. Both, F. Sato, A. R. Bellamy, and P. H. Atkinson.** 1985. Deletion into an NH_2-terminal hydrophobic domain results in secretion of rotavirus VP7, a resident endoplasmic reticulum membrane glycoprotein. J. Cell Biol. **101**:2199–2209.
260. **Porter, A. G., C. Barber, N. H. Carey, R. A. Hallewell, G. Threlfall, and J. S. Emtage.** 1979. Complete nucleotide sequence of an influenza virus haemagglutinin gene from cloned DNA. Nature (London) **282**:471–477.
261. **Pothier, P., E. Kohli, E. Drouet, and S. Ghim.** 1987. Analysis of

the antigenic sites on the major inner capsid protein (VP6) of rotaviruses using monoclonal antibodies. Ann. Inst. Pasteur/ Virol. **138**:285–295.

262. **Potter, A. A., G. Cox, M. Parker, and L. A. Babiuk.** 1987. The complete nucleotide sequence of bovine rotavirus C486 gene 4 cDNA. Nucleic Acids Res. **15**:4361.
263. **Powell, K. F. H., P. R. Gunn, and A. R. Bellamy.** 1988. Nucleotide sequence of bovine rotavirus genomic segment 10: an RNA encoding the viral non-structural glycoprotein. Nucleic Acids Res. **16**:763.
264. **Prasad, B. V. V., G. J. Wang, J. P. M. Clerx, and W. Chiu.** 1988. Three-dimensional structure of rotavirus. J. Mol. Biol. **199**:269–275.
265. **Quan, C. M., and F. W. Doane.** 1983. Ultrastructural evidence for the cellular uptake of rotavirus by endocytosis. Intervirology **20**:223–231.
266. **Ramig, R. F.** 1982. Isolation and genetic characterization of temperature-sensitive mutants of simian rotavirus SA11. Virology **120**:93–105.
267. **Ramig, R. F.** 1983. Genetic studies with simian rotavirus SA11, p. 321–327. *In* R. W. Compans and D. H. L. Bishop (ed.), Double-stranded RNA viruses. Elsevier Science Publishing, Inc., New York.
268. **Ramig, R. F.** 1983. Isolation and genetic characterization of temperature-sensitive mutants that define five additional recombination groups in simian rotavirus SA11. Virology **130**: 464–473.
269. **Ramig, R. F.** 1983. Factors that affect genetic interaction during mixed infection with temperature-sensitive mutants of simian rotavirus SA11. Virology **127**:91–99.
270. **Ramig, R. F.** 1988. The effects of host age, virus dose, and virus strain on heterologous rotavirus infection of suckling mice. Microb. Pathog. **4**:189–202.
271. **Ramig, R. F., and B. L. Petrie.** 1984. Characterization of temperature-sensitive mutants of simian rotavirus SA11: protein synthesis and morphogenesis. J. Virol. **49**:665–673.
272. **Ramig, R. F., S. K. Samal, and S. McConnell.** 1985. Genome RNAs of virulent and attenuated strains of bluetongue virus serotypes 10, 11, 13 and 17. Prog. Clin. Biol. Res. **178**:389–396.
273. **Ready, K. F. M., and M. Sabara.** 1987. *In vitro* assembly of bovine rotavirus nucleocapsid protein. Virology **157**:189–198.
274. **Richardson, C. D., and P. W. Choppin.** 1983. Oligopeptides that specifically inhibit membrane fusion by paramyxoviruses: studies on the site of action. Virology **131**:518–532.
275. **Richardson, C. D., A. Scheid, and P. W. Choppin.** 1980. Specific inhibition of paramyxovirus and myxovirus replication by oligopeptides with amino acid sequences similar to those at the N-termini of the F_1 or HA_2 viral polypeptides. Virology **105**:205–222.
276. **Richardson, M. A., A. Iwamoto, N. Ikegami, A. Nomoto, and Y. Furuichi.** 1984. Nucleotide sequence of the gene encoding the serotype-specific antigen of human (Wa) rotavirus: comparison with the homologous genes from simian SA11 and UK bovine rotaviruses. J. Virol. **51**:860–862.
277. **Richardson, S. C., L. E. Mercer, S. Sonza, and I. H. Holmes.** 1986. Intracellular localization of rotaviral proteins. Arch. Virol. **88**:251–264.
278. **Riepenhoff-Talty, M., T. Dharakul, E. Kowalski, S. Michalak, and P. L. Ogra.** 1987. Persistent rotavirus infection in mice with severe combined immunodeficiency. J. Virol. **61**:3345–3348.
279. **Riepenhoff-Talty, M., I. Uhnoo, P. Chegas, and P. Ogra.** 1989. Effect of nutritional deprivation on mucosal viral infections. Immunol. Invest. **18**(1–4):127–139.

279a. **Rixon, F., P. Taylor, and U. Desselberger.** 1984. Rotavirus RNA segments sized by electron microscopy. J. Gen. Virol. **65**:233–239.

280. **Rodger, S. M., and I. H. Holmes.** 1979. Comparison of the genomes of simian, bovine, and human rotaviruses by gel electrophoresis and detection of genomic variation among bovine isolates. J. Virol. **30**:839–846.
281. **Roseto, A., J. Esgaig, E. Delain, J. Cohen, and R. Scherrer.** 1979. Structure of rotaviruses as studied by the freeze-drying technique. Virology **98**:471–475.
282. **Roseto, A., R. Scherrer, J. Cohen, M. C. Guillemin, A. Charpilienne, C. Feynerol, and J. Peries.** 1983. Isolation and characterization of anti-rotavirus immunoglobulins secreted by cloned hybridoma cell lines. J. Gen. Virol. **64**:237–240.
283. **Ruiz, A. M., I. V. Lopez, S. Lopez, R. T. Espejo, and C. F. Arias.** 1988. Molecular and antigenic characterization of porcine rotavirus YM, a possible new rotavirus serotype. J. Virol. **62**:4331–4336.
284. **Rushlow, K., A. McNab, K. Olson, F. Maxwell, I. Maxwell, and G. Stiegler.** 1988. Nucleotide sequence of porcine rotavirus (OSU strain) gene segments 7, 8, and 9. Nucleic Acids Res. **16**:367.
285. **Sabara, M., L. A. Babiuk, J. Gilchrist, and V. Misra.** 1982. Effect of tunicamycin on rotavirus assembly and infectivity. J. Virol. **43**:1082–1090.
286. **Sabara, M., A. Barrington, and L. A. Babiuk.** 1985. Immunogenicity of a bovine rotavirus glycoprotein fragment. J. Virol. **56**:1037–1040.
287. **Sabara, M., J. E. Gilchrist, G. R. Hudson, and L. A. Babiuk.** 1985. Preliminary characterization of an epitope involved in neutralization and cell attachment that is located on the major bovine rotavirus glycoprotein. J. Virol. **53**:58–66.
288. **Sabara, M., K. F. M. Ready, P. J. Frenchick, and L. A. Babiuk.** 1987. Biochemical evidence for the oligomeric arrangement of bovine rotavirus nucleocapsid protein and its possible significance in the immunogenicity of this protein. J. Gen. Virol. **68**:123–133.
289. **Saif, L. J.** 1985. Passive immunity to coronavirus and rotavirus infections in swine and cattle: enhancement by maternal vaccination, p. 456–467. *In* S. Tzipori (ed.), Infectious diarrhoea in the young. Elsevier Biomedical Press, Amsterdam.
290. **Saif, L. J., K. L. Smith, B. J. Landmeier, E. H. Bohl, K. W. Theil, and D. A. Todhunter.** 1984. Immune response of pregnant cows to bovine rotavirus immunization. Am. J. Vet. Res. **45**:49–58.
291. **Saif, L. J., P. Weilnau, K. Miller, and L. Stitzlein.** 1987. Isotypes of intestinal and systemic antibodies in colostrum-fed and colostrum-deprived calves challenged with rotavirus. Adv. Exp. Med. Biol. **216B**:1815–1823.
292. **Saif, L. J., L. A. Terrett, K. L. Miller, and R. F. Cross.** 1988. Serial propagation of porcine group C rotavirus (pararotavirus) in a continuous cell line and characterization of the passaged virus. J. Clin. Microbiol. **26**:1277–1282.
293. **Sandino, A. M., M. Jashes, G. Faundez, and E. Spencer.** 1986. Role of the inner protein capsid on in vitro human rotavirus transcription. J. Virol. **60**:797–802.
294. **Sato, T., H. Suzuki, S. Kitaoka, T. Konno, and N. Ishida.** 1986. Patterns of polypeptide synthesis in human rotavirus infected cells. Arch. Virol. **90**:29–40.
295. **Schiff, L. A., M. L. Nibert, M. S. Co, E. G. Brown, and B. N. Fields.** 1988. Distinct binding sites for zinc and double-stranded RNA in the reovirus outer capsid protein σ3. Mol. Cell. Biol. **8**:273–283.
296. **Schmaljohn, A. L., K. M. Kokubun, and G. A. Cole.** 1983. Protective monoclonal antibodies define maturational and pH-dependent antigenic changes in Sindbis virus E_1 glycoprotein. Virology **130**:144–154.
297. **Schroeder, B. A., J. E. Street, J. Kalmakoff, and A. R. Bellamy.** 1982. Sequence relationships between the genome segments of human and animal rotavirus strains. J. Virol. **43**:379–385.
298. **Sethi, S. K., D. M. Olive, O. O. Strannegard, and W. Al-Nakib.** 1988. Molecular epidemiology of human rotavirus infections based on genome segment variations in viral strains. J. Med. Virol. **26**:249–259.
299. **Shahrabadi, M. S., L. A. Babiuk, and P. W. K. Lee.** 1987. Further analysis of the role of calcium in rotavirus morphogenesis. Virology **158**:103–111.
300. **Shahrabadi, M. S., and P. W. K. Lee.** 1986. Bovine rotavirus maturation is a calcium-dependent process. Virology **152**: 298–307.
301. **Shaw, R. D., K. J. Fong, G. A. Losonsky, M. M. Levine, Y. Maldonado, R. Yolken, J. Flores, A. Z. Kapikian, P. T. Vo, and**

H. B. Greenberg. 1987. Epitope-specific immune responses to rotavirus vaccination. Gastroenterology **93**:941–950.
302. **Shaw, R. D., E. M. Mackow, M. L. Dyall-Smith, I. Lazdins, I. H. Holmes, and H. B. Greenberg.** 1988. Serotypic analysis of VP3 and VP7 neutralization escape mutants of rhesus rotavirus. J. Virol. **62**:3509–3512.
303. **Shaw, R. D., D. L. Stoner-Ma, M. K. Estes, and H. B. Greenberg.** 1985. Specific enzyme-linked immunoassay for rotavirus serotypes 1 and 3. J. Clin. Microbiol. **22**:286–291.
304. **Shaw, R. D., P. T. Vo, P. A. Offit, B. S. Coulson, and H. B. Greenberg.** 1986. Antigenic mapping of the surface proteins of rhesus rotavirus. Virology **155**:434–451.
305. **Singh, N., M. M. Sereno, J. Flores, and A. Z. Kapikian.** 1983. Monoclonal antibodies to subgroup 1 rotavirus. Infect. Immun. **42**:835–837.
306. **Skehel, J. J., D. J. Stevens, R. S. Daniels, A. R. Douglas, M. Knossow, and I. A. Wilson.** 1984. A carbohydrate side-chain on hemagglutinin of the Hong Kong influenza viruses inhibits recognition by a monoclonal antibody. Proc. Natl. Acad. Sci. USA **81**:1779–1783.
307. **Smith, M. L., I. Lazdins, and I. H. Holmes.** 1980. Coding assignments of double-stranded RNA segments of SA11 rotavirus established by in vitro translation. J. Virol. **33**:976–982.
308. **Snodgrass, D. R., K. J. Fahey, P. W. Wells, I. Campbell, and A. Whitelaw.** 1980. Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. Infect. Immun. **28**:344–349.
309. **Snodgrass, D. R., A. J. Herring, I. Campbell, J. M. Inglis, and F. D. Hargreaves.** 1984. Comparison of atypical rotaviruses from calves, piglets, lambs and man. J. Gen. Virol. **65**:909–914.
310. **Snodgrass, D. R., C. K. Ojey, I. Campbell, and A. J. Herring.** 1984. Bovine rotavirus serotypes and their significance for immunization. J. Clin. Microbiol. **20**:342–346.
311. **Soler, C., C. Musalem, M. Lorono, and R. T. Espejo.** 1982. Association of viral particles and viral proteins with membranes in SA11-infected cells. J. Virol. **44**:983–992.
312. **Sonza, S., A. M. Breschkin, and I. H. Holmes.** 1983. Derivation of neutralizing monoclonal antibodies against rotavirus. J. Virol. **45**:1143–1146.
313. **Stacy-Phipps, S., and J. T. Patton.** 1987. Synthesis of plus- and minus-strand RNA in rotavirus-infected cells. J. Virol. **61**: 3479–3484.
314. **Steinhoff, M. C.** 1980. Rotavirus: the first five years. J. Pediatr. **96**:611–622.
315. **Stirzaker, S. C., and G. W. Both.** 1989. The signal peptide of the rotavirus glycoprotein is essential for its retention in the ER as an integral membrane protein. Cell **56**:741–747.
316. **Stirzaker, S. C., P. L. Whitfeld, D. L. Christie, A. R. Bellamy, and G. W. Both.** 1987. Processing of rotavirus glycoprotein VP7: implications for the retention of the protein in the endoplasmic reticulum. J. Cell Biol. **105**:2897–2903.
317. **Streckert, H.-J., H. Brussow, and H. Werchau.** 1988. A synthetic peptide corresponding to the cleavage region of VP3 from rotavirus SA11 induces neutralizing antibodies. J. Virol. **62**:4265–4269.
318. **Streckert, H.-J., B. Grunert, and H. Werchau.** 1986. Antibodies specific for the carboxy-terminal region of the major surface glycoprotein of simian rotavirus (SA11) and human rotavirus (Wa). J. Cell. Biochem. **30**:41–49.
319. **Su, C., Y. Wu, H. Shen, D. Wang, Y. Chen, D. Wu, L. He, and Z. Yang.** 1986. An outbreak of epidemic diarrhoea in adults caused by a new rotavirus in Anhui province of China in the summer of 1983. J. Med. Virol. **19**:167–173.
320. **Suzuki, H., G.-M. Chen, T. Hung, G. M. Beards, D. W. G. Brown, and T. H. Flewett.** 1987. Effects of two negative staining methods on the Chinese atypical rotavirus. Arch. Virol. **94**:305–308.
321. **Suzuki, H., S. Kitaoka, T. Konno, T. Sato, and N. Ishida.** 1985. Two modes of human rotavirus entry into MA104 cells. Arch. Virol. **85**:25–34.
322. **Suzuki, H., T. Sato, T. Konno, S. Kitaoka, T. Ebina, and N. Ishida.** 1984. Effect of tunicamycin on human rotavirus morphogenesis and infectivity. Arch. Virol. **81**:363–369.
323. **Svensson, L., L. Grahnquist, C.-A. Pettersson, M. Grandien, G. Stintzing, and H. B. Greenberg.** 1988. Detection of human rotaviruses which do not react with subgroup I- and II-specific monoclonal antibodies. J. Clin. Microbiol. **26**:1238–1240.
324. **Svensson, L., H. Sheshberadaran, S. Vene, E. Norrby, M. Grandein, and G. Wadell.** 1987. Serum antibody responses to individual viral polypeptides in human rotavirus infections. J. Gen. Virol. **68**:643–651.
325. **Tanaka, T. N., M. E. Conner, D. Y. Graham, and M. K. Estes.** 1988. Molecular characterization of three rabbit rotavirus strains. Arch. Virol. **98**:253–265.
326. **Taniguchi, K., Y. Hoshino, K. Nishikawa, K. Y. Green, W. L. Maloy, Y. Morita, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1988. Cross-reactive and serotype-specific neutralization epitopes on VP7 of human rotavirus: nucleotide sequence analysis of antigenic mutants selected with monoclonal antibodies. J. Virol. **62**:1870–1874.
327. **Taniguchi, K., W. L. Maloy, K. Nishikawa, K. Y. Green, Y. Hoshino, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1988. Identification of cross-reactive and serotype specific neutralization epitopes on VP3 of human rotavirus. J. Virol. **62**:2421–2426.
328. **Taniguchi, K., Y. Morita, T. Urasawa, and S. Urasawa.** 1987. Cross-reactive neutralization epitopes on VP3 of human rotavirus: analysis with monoclonal antibodies and antigenic variants. J. Virol. **61**:1726–1730.
329. **Taniguchi, K., S. Urasawa, and T. Urasawa.** 1985. Preparation and characterization of neutralizing monoclonal antibodies with different reactivity patterns to human rotaviruses. J. Gen. Virol. **66**:1045–1053.
330. **Taniguchi, K., T. Urasawa, Y. Morita, H. B. Greenberg, and S. Urasawa.** 1987. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J. Infect. Dis. **155**:1159–1166.
331. **Taniguchi, K., T. Urasawa, and S. Urasawa.** 1986. Reactivity patterns to human rotavirus strains of a monoclonal antibody against VP2, a component of the inner capsid of rotavirus. Arch. Virol. **87**:135–141.
332. **Tao, H.** 1988. Rotavirus and adult diarrhea. Adv. Virus Res. **35**:193–218.
332a. **Taylor, P., F. Rixon, and U. Desselberger.** 1985. Rise per base pair in helices of double-stranded rotavirus RNA determined by electron microscopy. Virus Res. **2**:175–182.
333. **Theil, K. W., and C. M. McCloskey.** 1988. Partial characterization of a bovine group A rotavirus with a short genome electropherotype. J. Clin. Microbiol. **26**:1094–1099.
334. **Thouless, M. E., R. F. DiGiacomo, B. J. Deeb, and H. Howard.** 1988. Pathogenicity of rotavirus in rabbits. J. Clin. Microbiol. **26**:943–947.
335. **Thouless, M. E., R. F. DiGiacomo, and D. S. Neuman.** 1986. Isolation of two lapine rotaviruses: characterization of their subgroup, serotype and RNA electropherotypes. Arch. Virol. **89**:161–170.
336. **Totterdell, B. M., J. E. Banatvala, I. L. Chrystie, G. Ball, and W. D. Cubitt.** 1988. Systemic lymphoproliferative responses to rotavirus. J. Med. Virol. **25**:37–44.
337. **Treanor, J., and R. Dolin.** 1984. Viral infections of the gastrointestinal tract, p. 369–397. *In* G. J. Gallasso, T. C. Merigan, and R. A. Buchanan (ed.), Antiviral agents and viral diseases of man. Raven Press, New York.
338. **Tufvesson, B., S. Polberger, L. Svanberg, and T. Sveger.** 1986. A prospective study of rotavirus infections in neonatal and maternity wards. Acta Pediatr. Scand. **75**:211–215.
339. **Tursi, J. M., M. J. Albert, and R. F. Bishop.** 1987. Production and characterization of neutralizing monoclonal antibody to a human rotavirus strain with a "super-short" RNA pattern. J. Clin. Microbiol. **25**:2426–2427.
340. **Unicomb, L. E., B. S. Coulson, and R. F. Bishop.** 1989. Experience with an enzyme immunoassay for serotyping human group A rotaviruses. J. Clin. Microbiol. **27**:586–588.
341. **Urasawa, S., T. Urasawa, and K. Taniguchi.** 1986. Genetic reassortment between two human rotaviruses having different

serotype and subgroup specificities. J. Gen. Virol. **67**:1551–1559.

342. **Urasawa, S., T. Urasawa, K. Taniguchi, Y. Morita, N. Sakurada, Y. Saeki, O. Morita, and S. Hasegawa.** 1988. Validity of an enzyme-linked immunosorbent assay with serotype specific monoclonal antibodies for serotyping human rotavirus in stool specimens. Microbiol. Immunol. **32**:699–708.

342a. **Urasawa, S., T. Urasawa, K. Taniguchi, F. Wakasugi, N. Kobayashi, S. Chiba, N. Sakurada, M. Morita, O. Morita, M. Tokieda, H. Kawamoto, Y. Minekawa, and M. Ohseto.** 1989. Survey of human rotavirus serotypes in different locales in Japan by enzyme-linked immunosorbent assay with monoclonal antibodies. J. Infect. Dis. **160**:44–51.

342b. **Ushijima, H., H. Honma, A. Mukoyama, T. Shinozaki, Y. Fujita, M. Kobayashi, M. Ohseto, S. Morikawa, and T. Kitamura.** 1989. Detection of group C rotaviruses in Tokyo. J. Med. Virol. **27**:299–303.

343. **Vesikari, T.** 1985. Progress in rotavirus vaccination. Pediatr. Infect. Dis. J. **4**:612–614.

344. **Vial, P. A., K. L. Kotloff, and G. A. Losonsky.** 1988. Molecular epidemiology of rotavirus infection in a room for convalescing newborns. J. Infect. Dis. **157**:668–673.

345. **Ward, C. W., A. A. Azad, and M. L. Dyall-Smith.** 1985. Structural homologies between RNA gene segments 10 and 11 from UK bovine, simian SA11, and human Wa rotaviruses. Virology **144**:328–336.

346. **Ward, C. W., T. C. Elleman, A. A. Azad, and M. L. Dyall-Smith.** 1984. Nucleotide sequence of gene segment 9 encoding a nonstructural protein of UK bovine rotavirus. Virology **134**:249–253.

347. **Ward, R. L., D. R. Knowlton, and H. B. Greenberg.** 1988. Phenotypic mixing during coinfection of cells with two strains of human rotavirus. J. Virol. **62**:4358–4361.

348. **Ward, R. L., D. R. Knowlton, and P. L. Hurst.** 1988. Reassortant formation and selection following coinfection of cultured cells with subgroup 2 human rotaviruses. J. Gen. Virol. **69**: 149–162.

349. **Welch, S.-K. W., S. E. Crawford, and M. K. Estes.** 1989. The rotavirus SA11 genome segment 11 protein is a nonstructural phosphoprotein. J. Virol. **63**:3974–3982.

350. **Whitfeld, P. L., C. Tyndall, S. C. Stirzaker, A. R. Bellamy, and G. W. Both.** 1987. Location of sequences within rotavirus SA11 glycoprotein VP7 which direct it to the endoplasmic reticulum. Mol. Cell. Biol. **7**:2491–2497.

351. **W.H.O. Programme for Control of Diarrhoeal Diseases.** 1987. Sixth Programme Report, 1986–1987. World Health Organization, Geneva.

352. **W.H.O. Steering Committee of the Scientific Working Group on Viral Diarrhoeas.** 1984. Nomenclature of human rotaviruses: designation of subgroups and serotypes. Bull. W.H.O. **62**: 501–503.

353. **Woode, G. N., J. Bridger, G. A. Hall, J. M. Jones, and G. Jackson.** 1976. The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. Med. Microbiol. **9**:203–209.

354. **Woode, G. N., N. E. Kelso, T. F. Simpson, S. K. Gaul, L. E. Evans, and L. Babiuk.** 1983. Antigenic relationships among some bovine rotaviruses: serum neutralization and cross-protection in gnotobiotic calves. J. Clin. Microbiol. **18**:358–364.

355. **Woode, G. N., S. Zheng, B. I. Rosen, N. Knight, N. E. K. Gourley, and R. F. Ramig.** 1987. Protection between different serotypes of bovine rotavirus in gnotobiotic calves: specificity of serum antibody and coproantibody responses. J. Clin. Microbiol. **25**:1052–1058.

356. **Yolken, R. H., S. B. Wee, J. Eiden, J. Kinney, and S. Vonderfecht.** 1988. Identification of a group-reactive epitope of group B rotaviruses recognized by monoclonal antibody and application to the development of a sensitive immunoassay for viral characterization. J. Clin. Microbiol. **26**:1853–1858.

357. **Yolken, R. H., R. Willoughby, S.-B. Wee, R. Miskuff, and S. Vonderfecht.** 1987. Sialic acid glycoproteins inhibit *in vitro* and *in vivo* replication of rotaviruses. J. Clin. Invest. **79**:148–154.

358. **Zhaori, G., M. Sun, and P. L. Ogra.** 1988. Characteristics of the immune response to poliovirus virion polypeptides after immunization with live or inactivated polio vaccines. J. Infect. Dis. **158**:160–165.